ARCHIVES OF PATHOLOGY

VOLUME 21 APRIL 1936 NUMBER 4

ATHEROSCLEROSIS

SPECIAL CONSIDERATION OF AORTIC LESIONS

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The results of a study of the lesions of coronary sclerosis in human beings [1] made it evident that in youth atherosclerosis of the coronary arteries is marked by an increase in the amount of subendothelial connective tissue, which narrows the lumen of the vessels. This growth is associated with the presence of lipoid cells, which are observed in small groups in the new fibrous tissue. In early youth the connective tissue is loose-textured, with little or no formation of collagen. In the fourth decade collagen formation begins to be evident in the lesions, with the production of true scar tissue. This is more manifest in the fifth decade.

In old age, on the other hand, while scar tissue which has persisted from an earlier period is often seen in the intima, the characteristic lesion is the accumulation of lipoid cells in masses with so little fibrous tissue support and so little provision for the nutrition of the cell masses that widespread necrosis of these cells occurs. In this way atheromatous "abscesses" are formed. The massing of lipoid cells and the atheromatous "abscesses" resulting from their necrosis are characteristic of the coronary lesions in older persons.

Occlusion of the lumen in youth is due to thrombosis, following in most cases acute fibrinous necrosis arising in the subendothelial layer and extending to the endothelium. Occlusion of the lumen in old age is due to the rupture of an atheromatous "abscess" into the lumen.

In a considerable percentage of cases death occurs without complete obstruction of the narrowed lumen and is apparently related to spasm of the vessel wall.[2] This is particularly common in cases of hypertension with arteriosclerosis.

From the Medical Examiner Service, Suffolk County.

Aided by a grant from the Committee on Scientific Research of the American Medical Association.

This work formed the basis of the Eleventh Ludvig Hektoen Lecture of the Frank Billings Foundation of the Institute of Medicine of Chicago (J. A. M. A. **105**:475, 1935; Proc. Inst. Med. Chicago **10**:289, 1935).

1. Leary, T.: Arch. Path. **17**:453, 1934.
2. Leary, T.: Am. Heart J. **10**:338, 1935.

Experimental atherosclerosis of the coronary arteries in young rabbits [1] is marked by fibrosis, as is atherosclerosis of these arteries in young human beings. Only exceptionally after the administration of massive doses of cholesterol is atheromatous "abscess" formation seen in the arteries of young rabbits. Fibrosis is therefore the reaction of youth to the presence of cholesterol and not a reaction of species.

While the evidence in these studies indicated that fibrosis occurs in youth and does not occur in old age, the reason for the differences in the reactions at these ages was not made clear. Resort was then had to a detailed study of lesions in the aorta. Aortic sclerosis is the most spectacular and familiar form of the human disease. It is marked by so great a variety of lesions that it is difficult to believe a single agency can be responsible for their production.

MATERIAL AND METHODS

Material covering, as far as possible, the whole gamut of the diverse aortic lesions was obtained at autopsies in my service as medical examiner, supplemented by aortas from the pathologic service of the Boston City Hospital, obtained through the courtesy of Dr. Frederic Parker Jr., and material from the Children's Hospital, secured through the courtesy of Dr. Sidney Farber.

The lesions of the experimental disease were produced in rabbits fed cholesterol in sunflower-seed oil for from seven to nine months, some of which were allowed to live following cessation of feeding for from five to nine months. The feeding procedure followed the method described in an earlier paper.[1]

Most of the material was studied in frozen sections, after fixation in solution of formaldehyde, or in fresh sections. The lipoids were stained with sudan IV; the nuclei, with hematoxylin.

Specimens fixed in formaldehyde and in Zenker's fluid were stained with hematoxylin and eosin, methylene blue and eosin, and Mallory's aniline blue connective tissue and phosphotungstic acid stains.

Polariscopic Study of Cholesterol.—The anisotropism of cholesterol and its esters makes it possible to follow this substance and its compounds in the tissues to a degree. Cholesterol occurs in the lesions of atherosclerosis in two forms:

1. Cholesterol esters. The lipoid droplets, as viewed through the polariscope, exhibit Maltese cross markings, producing a tetrad-like appearance. These are the "fluid crystals" of Lehman. Figure 1 *A* is from a smear of the surface layer of scrapings of the adrenal cortex suspended in saline solution and centrifugated. Similar preparations may be made from early aortic lesions. Figure 1 *B* is from a fresh section of an early atherosclerotic lesion. Where the cells have ruptured the fluid crystals are evident.

2. Free cholesterol in solid crystals. These occur when, as the result of necrosis, cholesterol is precipitated from the esters, as in the contents of atheromatous "abscesses." They appear as typical plates, usually fused into masses (fig. 1 *C*). No other known agents than cholesterol are likely to give rise to these pictures.

The cholesterol in the ester form is in such unstable combination that fixation in formaldehyde tends to be followed by splitting of the esters and precipitation in the familiar plates, which often fuse. Heating tends to solution of the crystals, with loss of anisotropism. Cold causes the crystals to reappear, though very low temperatures are often necessary in order to bring back all the crystals. Staining with sudan tends to produce solution of some of the smaller crystals.

AORTIC LESIONS

Gross Appearance.—Grossly the aorta may show:

(*a*) Flat pale yellow streaks or bands without projection, some with raised bright yellow edges which suggest an advancing border of the lesion or with bright yellow lines or points. This type of lesion may

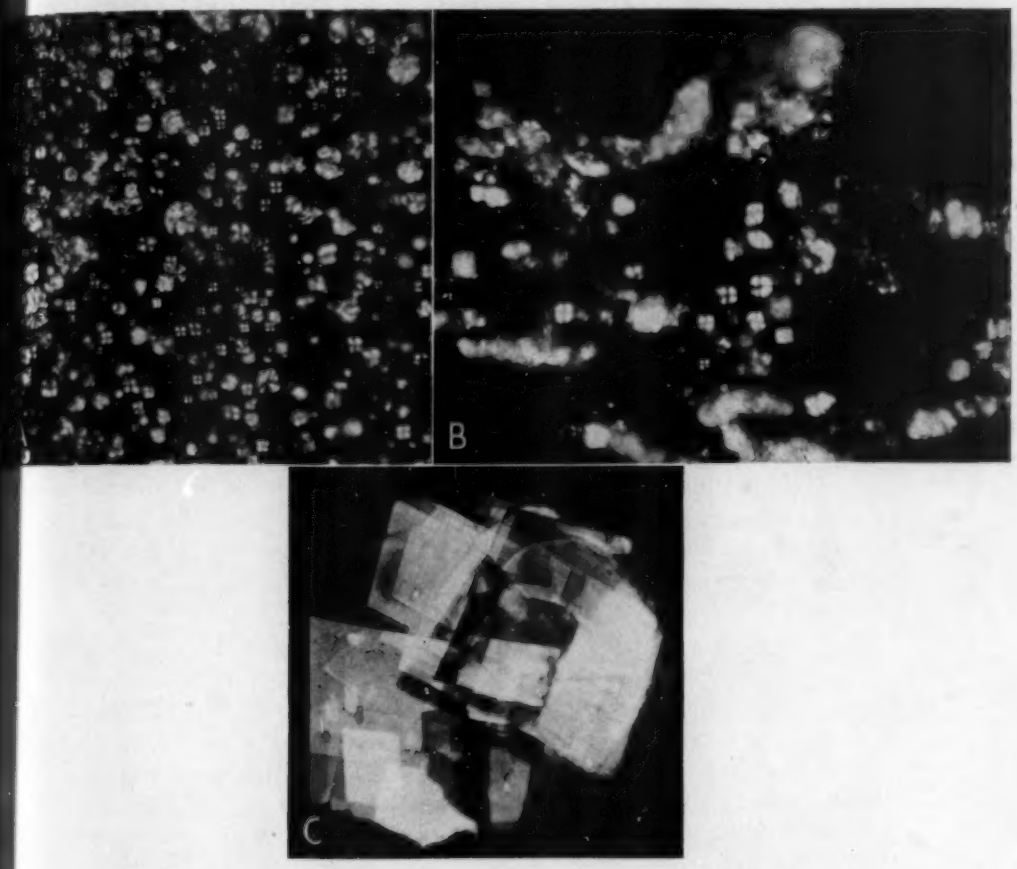

Fig. 1.—*A*, cholesterol esters—"fluid crystals"—in the adrenal cortex; × 200. *B*, aortic atherosclerosis. "Fluid crystals" in a frozen section; unstained; × 215. *C*, fused cholesterol crystal from an atheromatous "abscess"; × 115.

give rise to mosaic effects of grouped streaks, and fused lesions may cover large areas (figs. 1 *C* to 8, inclusive).

(*b*) Small bright yellow mounds, which with age tend to become paler. These are seen particularly in the ascending aortic arch (figs. 16 and 17).

(*c*) Large yellow or yellowish-white mounds, evidently arising by extension from smaller foci (too large to be shown in a single microscopic field).

(*d*) Pale yellow to white scarred nodules covered by a thin surface layer over a yellow base (figs. 9 and 10).

(*e*) White foci, apparently scarred throughout as viewed from the surface but showing on gross section a yellow base (figs. 11 and 12).

(*f*) Scars which on gross section appear to be pure white (fig. 13).

(*g*) Raised yellow foci covered with a thin surface layer which readily ruptures, disclosing yellow pultaceous material—atheromatous "abscesses" (figs. 14 and 15 *A*).

(*h*) Ruptured atheromatous "abscesses," pigmented because of contained clot (fig. 15 *B*) or forming frank ulcers, on which thrombosis may have occurred.

(i) Calcified plaques (figs. 18 and 19 *A*).

As in a kaleidoscope a limited number of elements produce the effect of infinite variety as their combinations change, so in the aorta modifications in the admixture of the lesions described give rise to a diversity of gross pictures which are bewildering on casual examination.

Microscopic Appearance.—I. Youth: The earliest aortic lesion in human beings is observed accidentally, usually in association with more advanced lesions. As seen in frozen sections there is a deposit of sudan-staining material (lipoid) in the form of fine droplets in the ground substance of the subendothelial layer. The ground substance is swollen. The lipoid is isotropic, i. e., has the character of neutral fat. After the fat has been extracted, as in paraffin embedding, sections disclose local changes in the subendothelial layer corresponding to those of mucoid degeneration. The fat is apparently deposited in subendothelial connective tissue which has undergone mucoid degeneration.

The next step is the appearance of macrophages in the surface layer under the endothelium. These cells are ovoid or globular, and the fat is diffused within them in the form of a very fine emulsion (fig. 2 *A*), just as it occurs normally in the cells of the adrenal cortex. Under the polariscope this lipoid is strongly anisotropic. Scrapings from the surface or the contents of cells ruptured in making fresh frozen sections reveal the typical tetrad-like fluid crystals of cholesterol esters (fig. 1 *B*).

Whether this difference in the polariscopic reaction of the intracellular lipoid as compared with the intercellular deposit is due to a selective separation of cholesterol esters which have been in solution in the subendothelial neutral fat or the formation of cholesterol esters from the cholesterol content of the neutral fat or whether it represents

a selective withdrawal of cholesterol esters from the circulating blood lipoid, without reference to the neutral fat already present in the swollen ground substance, remains a question. The difference in the polariscopic reaction may arise also from the invasion through the endothelium of monocytes carrying the lipoid. The free subendothelial lipoid in the ground substance tends to diminish in amount and ultimately to dis-

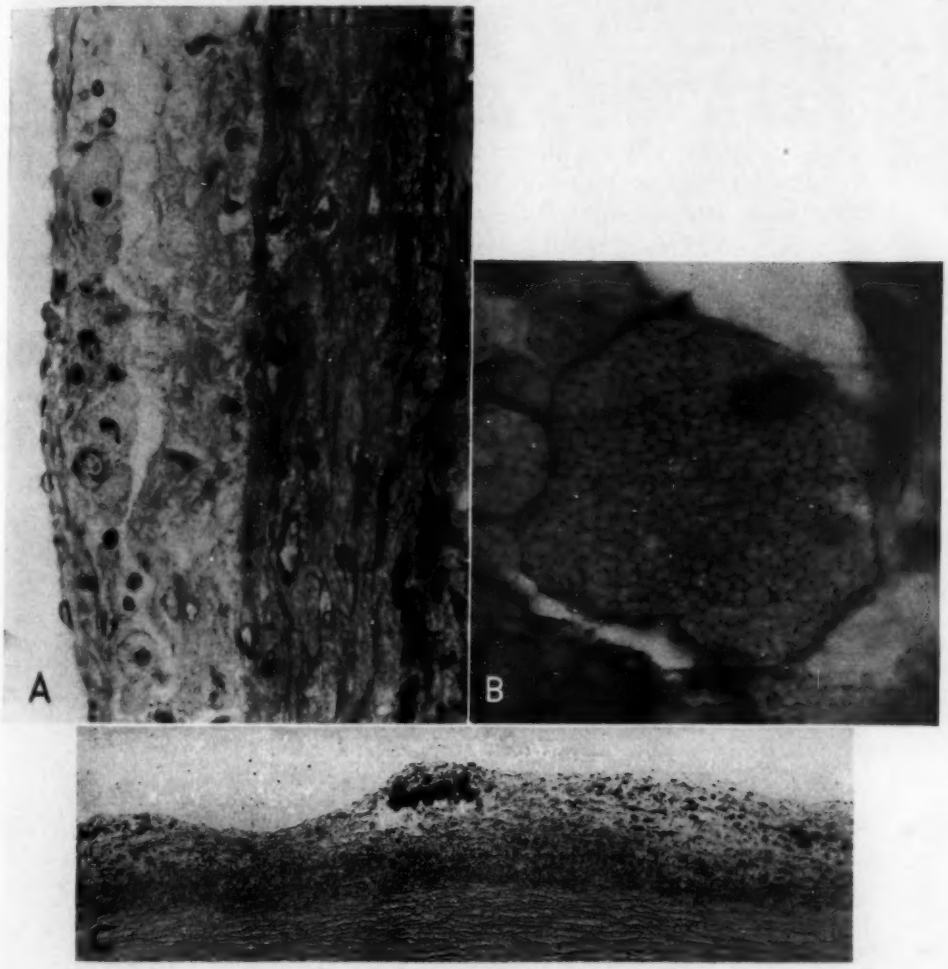

Fig. 2.—*A*, early aortic lesion with macrophages filled with lipoid, from a patient aged 14 years. Section fixed in Zenker's fluid and embedded in paraffin, stained with Mallory's aniline blue connective tissue stain; × 400. *B*, globular lipophage, focused to show the fine emulsion of lipoid. Section fixed in Zenker's fluid and embedded with paraffin, stained with Mallory's aniline blue connective tissue stain; × 970. *C*, aortic lesion from a patient aged 20, showing massed globular macrophages at one point. Reticular tissue with diffused branching lipophages. Frozen section stained with hematoxylin and sudan IV; × 50.

appear in many cases, though the disappearance occurs later than at this early stage.

Thus far the lesion has four outstanding characteristics: (1) mucoid degeneration of the ground substance, in which there is deposited (2) isotropic fat, and the appearance of (3) subendothelial macrophages which are filled with (4) anisotropic fat in fine emulsion.

In the young the common gross aortic lesion consists of pale yellow spots and bands with bright yellow raised points or lines. Microscopically the bright yellow points or lines are seen to owe their brighter color to focal accumulations of globular macrophages filled with lipoid and located at or near the surface of the lesion beneath the endothelium. The paler yellow portions of the lesion are made up of a loose reticular connective tissue through which branching cells of irregular shape, filled with lipoid in coarser drops, are dispersed singly (fig. 2*C*). There are added to the four characteristics described in the earlier lesion an increase in the amount of subendothelial connective tissue and the occurrence of a type of phagocytic cell of irregular shape and containing the lipoid in larger drops than in the first globular cell described.

The lesions now contain what appear to be two distinct types of phagocytic cells.

1. The first type to appear tends to remain a more or less globular cell, which may vary greatly in size and in which the lipoid is present in the form of cholesterol esters in fine emulsion distributed evenly in droplets of essentially equal size throughout the cytoplasm (fig. 2*B*), though in young cells the cytoplasm stains in the form of fine granules without very definite droplet formation of the lipoid. Compression of the cell by surrounding tissues may lead to an oval or elongated form, but the impression of a basically globular cell remains. This cell is ameboid, since though it appears first in the surface layer of the lesions directly beneath the endothelium, it may be seen later in the depths of relatively thick lesions. In the experimental rabbit cells of this type tend to make their way through the relatively thin walls of aortic branches, and perivascular lymphatics may be crowded with them. This cell belongs to the monocyte-histiocyte series and will be referred to for convenience as a globular lipophage, without prejudice as to its source. In the aortic lesions under study these cells tended to undergo necrobiosis, evidenced by disintegration of the cytoplasm and fusion of the lipoid into larger, irregular drops, associated with degeneration of the nucleus.

2. The second type of phagocytic cell is irregularly shaped and appears in the lesions only after a growth of subendothelial connective tissue has arisen. Cells of this type as seen in the frozen sections stained for fat have all the appearances of actively ameboid cells. They

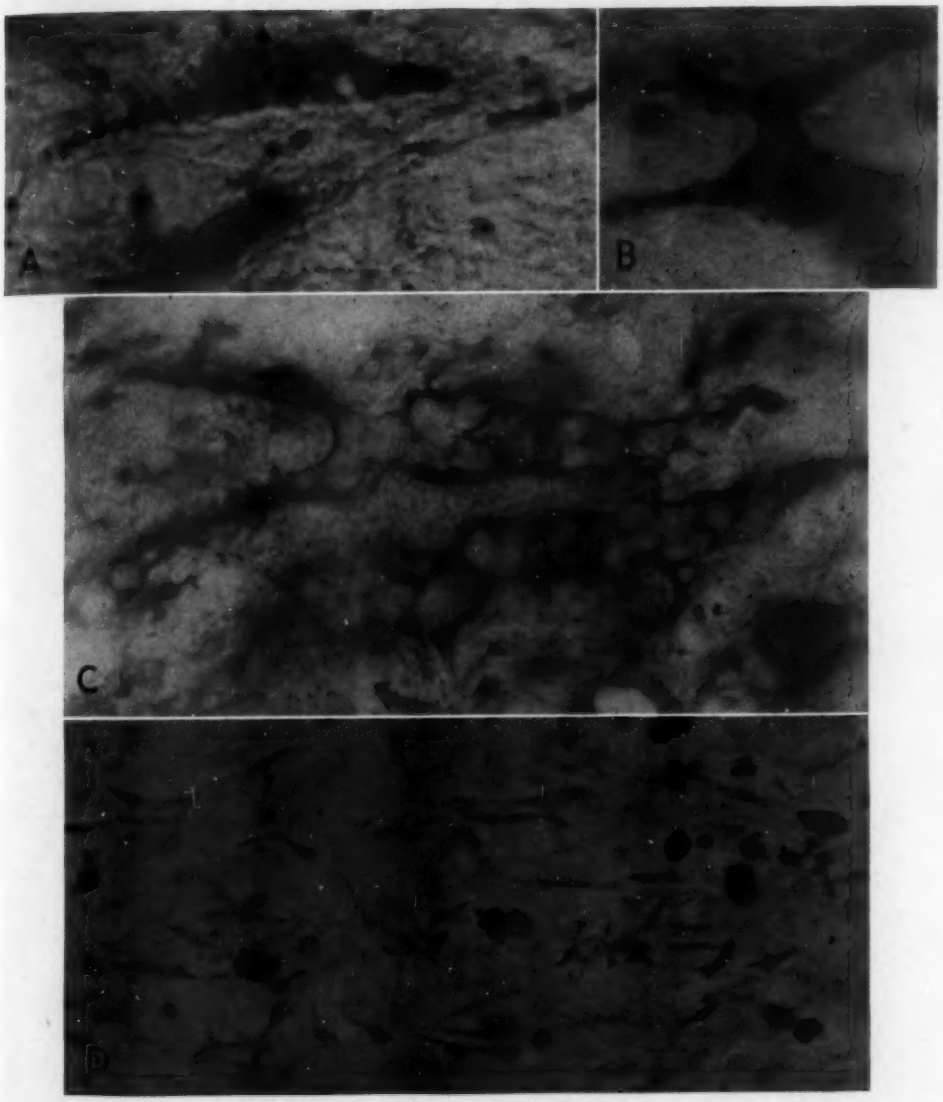

Fig. 3.—*A*, branching lipophage; × 1,152. *B*, × 1,240. Frozen sections stained with hematoxylin and sudan IV. *C*, fibrolipophages. Section fixed in Zenker's fluid and embedded in paraffin, stained with Mallory's aniline blue connective tissue stain; × 1,470. *D*, globular lipophages and spindle cell fibrolipophages. Practically every fibroblast in this region contains cholesterol. The patient was 6 months old. Frozen section stained with hematoxylin and sudan IV; × 250.

have branching processes, sometimes four or more, and sprawl through many planes of a section, so that adequate photomicrographs, necessarily limited to a single plane, are difficult or impossible to obtain. The lipoid is contained within them in larger drops than in cells of the type first described. The drops are irregular in size, and the cell processes contain smaller drops than the cell bodies (fig. 3 *A*). These cells were looked on as ameboid cells in my earlier description of the lesions, based on studies of frozen sections and sections stained with hematoxylin and eosin. Sections fixed in Zenker's fluid and stained by Mallory's aniline blue and phosphotungstic acid stains showed these cells to be fibroblasts, which form definite fibroglial fibrils (fig. 3 *C*). This cell will be referred to as a fibrolipophage.

The globular lipophage is not merely the first phagocytic cell to appear in the lesions; new crops of these cells appear in the layer underlying the endothelium as long as the lesion is progressive, i. e., as long as new deposits of cholesterol continue to be introduced. They may be spread in a continuous layer along the whole surface of the lesion in active processes. As the intake of cholesterol diminishes they are observed as focal collections in the surface layer. When this intake ceases, in the young the globular lipophages tend to become fewer in number and finally disappear (by degeneration ?), leaving the fibrolipophages the only cells in the lesions containing lipoid. There is a strong suggestion that these globular cells are monocytes which invade the subendothelial layer, already filled with lipoid.

In active lesions almost all the fibroblasts in the new connective tissue come to contain lipoid (fig. 3 *D*). Only an occasional cell is without lipoid. As the intake of lipoid at the surface ceases, the amount of lipoid within the fibrolipophages gradually becomes less. Lipoid then disappears from lesions which are regressing, first from cells along the edges of the lesions and later from the more active centers. Though degenerative changes in the globular lipophages are common in the lesions, no corresponding changes have been observed in the fibrolipophages. The fibrolipophages which have lost their lipoid contents revert to simple fibroblasts.

Polariscopically the fat in the fibrolipophages tends to occur in the form of solid crystals of cholesterol in formaldehyde-fixed, frozen sections of early lesions. This suggests the activities of cholesterase in an early stage of the process. The cells in the early stages are rich in anisotropic lipoid. As the lesions age, the lipoid within the fibrolipophages tends to become progressively less anisotropic. What anisotropism is evident in later stages is due to fine solid crystals of cholesterol, occurring singly or in small groups in some cells. When the intracellular lipoid has disappeared sudan-staining material may remain for a time in the lesion in the form of infinitely fine droplets

(Bloor's fat dust) lying along the wavy fibrillae in the interstitial spaces. This fat dust is isotropic.

Figure 4 is from a frozen section of an aortic lesion in a girl of 9 years who died as the result of intestinal obstruction due to volvulus. It will be observed that the subendothelial tissue, apart from the surface layer, which contains globular lipophages, shows series of elongated and branching cells in which the fat is contained in definite drops. What appear to be free drops are in the processes of cells the bodies of

Fig. 4.—Globular lipophages near the surface, with fibrolipophages below. The patient was 9 years old. Frozen section stained with hematoxylin and sudan IV; × 380.

which lie without the plane of the section. Figure 5 is a polariscopic photomicrograph of an unstained neighboring section of the same lesion. It will be observed that while the lipoid in the surface globular lipophages is markedly anisotropic and occurs as fluid crystals of cholesterol esters, the lipoid in the fibrolipophages is in the form of fine solid crystals, which are few in number.

An effort to show this change, more graphically if more diagrammatically, is recorded in figure 6. The section was stained with sudan IV so that the fibrolipophages could be identified. The photograph was made through a polarizing microscope, but the analyzer was turned so that enough direct light was admitted to permit of reproducing an image. The procedure was faulty, in that sudan staining tends to dissolve small crystals of cholesterol and the direct light blurred the crystalline cholesterol masses in the globular lipophages. In spite of

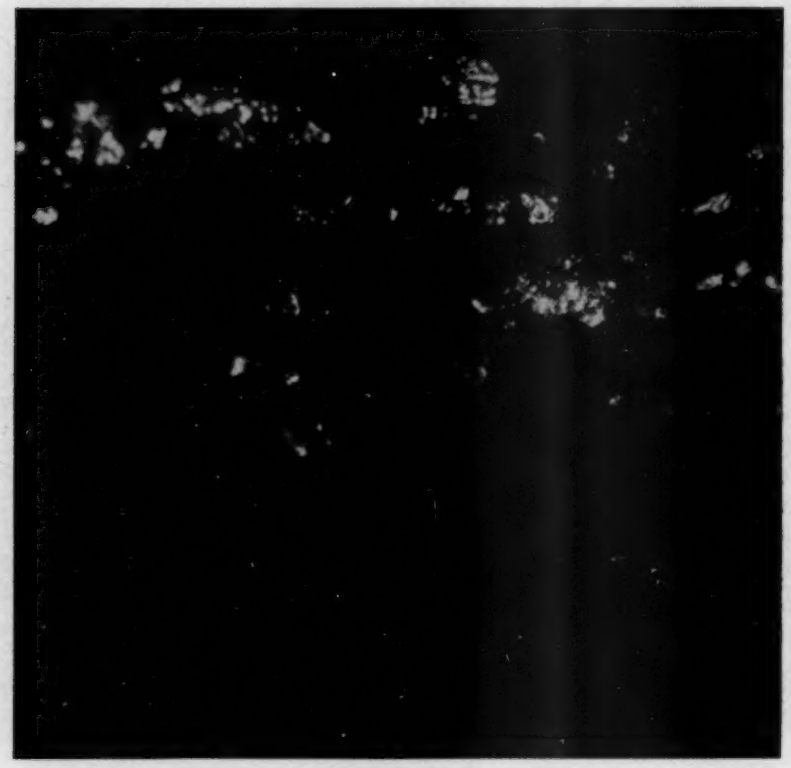

Fig. 5.—Polariscopic photograph of a frozen section adjacent to that shown in figure 4. Unstained; × 380.

these limitations, the contrast between the markedly anisotropic lipoid in the globular lipophages and the almost complete loss of anisotropism in the fibrolipophages is manifest. Possible errors in interpretation can be checked by reference to figures 4 and 5.

The processes described in aortic lesions up to this point include, in addition to the six characteristics referred to, changes which appear to be largely limited to the functioning of the fibrolipophages. In these cells or in relation to their phagocytosis of lipoid the cholesterol esters

undergo splitting, the cholesterol appearing in solid crystals. The lipoid next loses its anisotropism and finally disappears from the lesion. Then follows repair of the process.

The young fibroblastic tissue of the lesions forms little or no collagen in youth. The tissue remains loose—reticular—and repair is followed by minimal scarring. Even in early youth the process of removal of fat may meet with difficulty if the amount of deposited lipoid is great. The massive lesion shown in figure 7 was from the aorta of an infant

Fig. 6.—Polariscopic photograph of a section of the lesion shown in figures 4 and 5 with some direct light admitted. Frozen section stained with hematoxylin and sudan IV; × 410.

of 6 months who died of pneumonia. The deposit of lipoid was so great, including invasion of the media by globular lipophages, that all the lipoid was not removed. Lipoid masses are still evident in the deep layers of the lesion. The character of the reticular tissue is well shown. Final healing of the lesions in youth, because of the essential absence of collagen formation, is associated with minimal scarring, as is shown in figue 8 *A*, from the aorta of another infant of 6 months.

To recapitulate, the lesions in youth show a sequence of development as follows:

1. Swelling and mucoid degeneration of the ground substance of the subendothelial layer of the intima.

2. Deposit of isotropic fat in the swollen ground substance.

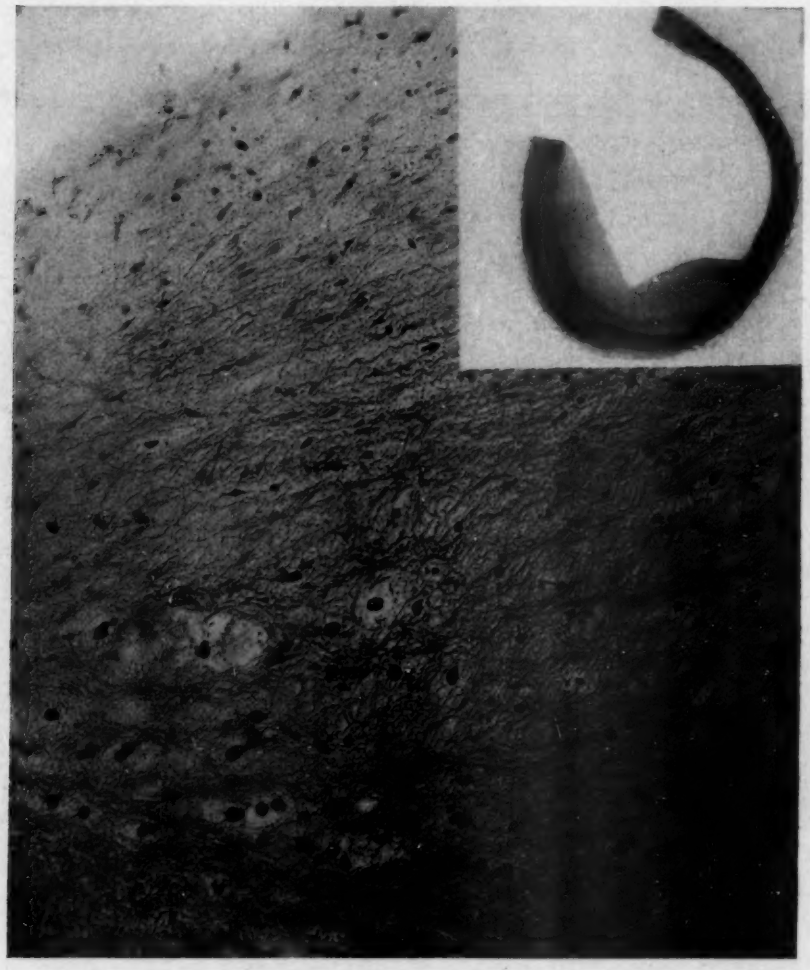

Fig. 7.—Massive lesion. The patient was 6 months old. A photograph of the lesion is inserted in the upper right corner. The detail is shown in the rest of the figure. Frozen section stained with hematoxylin and sudan IV; ×136; insert, × 4.5.

3. Appearance of globular lipophages in the layer beneath the endothelium.

4. Surcharging of these cells with anisotropic fat (cholesterol esters) in fine emulsion.

5. Stimulation of the subendothelial connective tissue.

6. Occurrence of a second type of branching phagocyte—fibrolipophage—containing lipoid (anisotropic) in larger drops of irregular size.

7. Splitting of the cholesterol esters apparently within or in relation to the formation of these cells.

8. Disappearance of globular lipophages from the lesions.

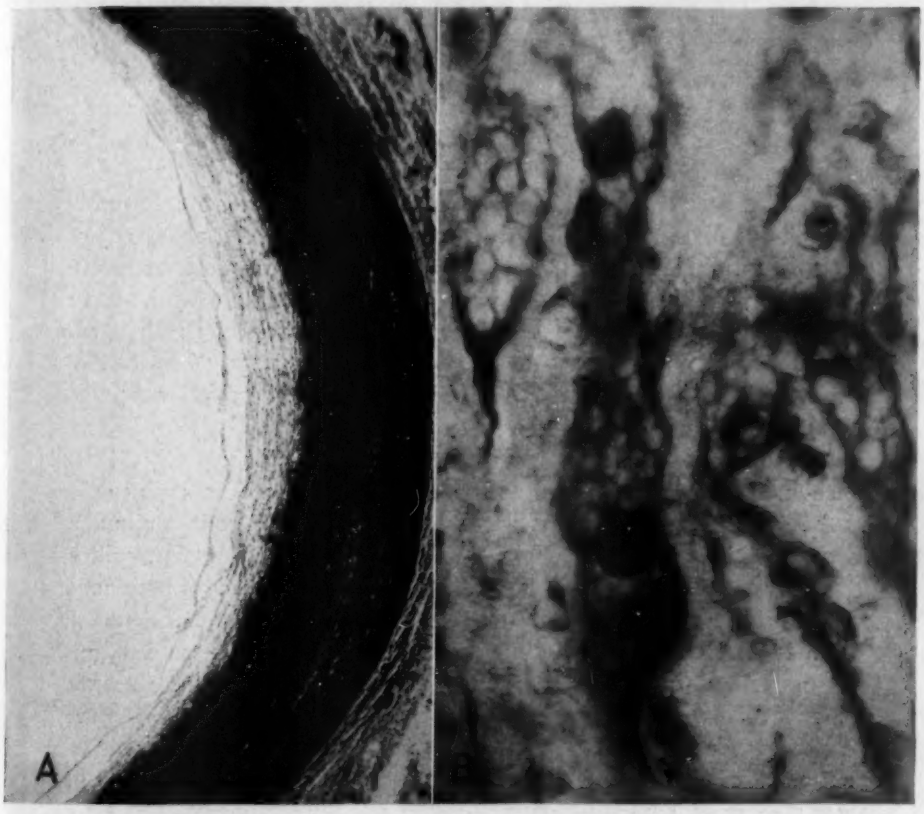

Fig. 8.—*A*, healed lesion; × 28. The patient was 6 months old. *B*, a group of fibrolipophages; × 1,470. Sections fixed in Zenker's fluid and embedded in paraffin, stained with Mallory's aniline blue connective tissue stain.

9. Loss of anisotropism of the lipoid in fibrolipophages.

10. Disappearance of the lipoid from the lesions—the fibrolipophages reverting to simple fibroblasts.

11. Repair, with little or no collagen formation and minimal scarring.

The fibrolipophage appears in the lesions later than the globular lipophage, and on its activities seem to depend the removal of the cholesterol and the repair of the lesions. The changes which the cholesterol undergoes in the fibrolipophage have all the character of a metabolism of the substance.

The suggestion from the study of the lesions is that globular lipophages are being formed or deposited beneath the endothelium in continuous series during the active phases of the lesions. As they invade the deeper layers among the fibrolipophages they appear to undergo necrosis, and the lipoid so freed is apparently picked up by fibrolipophages which bring about its elimination from the lesions preliminary to repair. Almost all the fibroblasts in progressive processes contain lipoid, so that many fields show the tissues apparently bubbling with intracellular fat (fig. 8*B*).

II. Middle Period of Life: As the body ages, collagen formation begins to manifest itself in the lesions of atherosclerosis, aortic as well as coronary. With the production of collagen, the new tissue as it ages tends to show all the characteristics of scar tissue. The older the lesion, the denser becomes the scar tissue. The mutations in color which the lesions undergo as they age—the changes from brilliant chrome yellow to silvery white—are dependent on the relative amounts of lipoid cells and scar tissue and their location in the lesion. If fresh lipoid is being deposited in the surface layers and globular lipoid cells are abundant toward the surface, yellow will be the dominating color. If the surface layer is almost purely scar tissue and lipoid is abundant in the depths, the lesion will be white grossly, and section will be necessary to disclose the yellow base. In old repaired lesions both actual and cut surfaces will be white.

A lesion showing beginning collagen formation is seen in figure 9*A*, a section from a yellow nodule in the transverse aortic arch of a man of 36 years. The lesion is rich in globular lipophages at and near the surface. Fibrolipophages are present in considerable numbers in the layer below the massed globular cells to the left, but they lie in spaces between bands of collagenous connective tissue. The deeper layers on the right exhibit necrosis, which includes the surface layers of the media. Figure 9*B* is a polariscopic photograph of an unstained section of this lesion. The globular lipophages in this section are more sharply limited to the subendothelial layer except near the ends. Few crystals are present in the deeper intimal layers, but the region of necrosis, including the surface layer of the media, is indicated by series of shadowy gray-white points.

A section from a yellowish-white nodule from a man 46 years of age is shown in figure 10*A*. The surface and the edges of the nodule

are made up of collagenous connective tissue. The mass of the mound consists of necrotic detritus. Scar tissue containing globular lipophages occupy the surface layer. In the depths of this layer globular lipophages are undergoing necrosis and apparently falling into the mass of amorphous necrotic material making up the base of the lesion. A

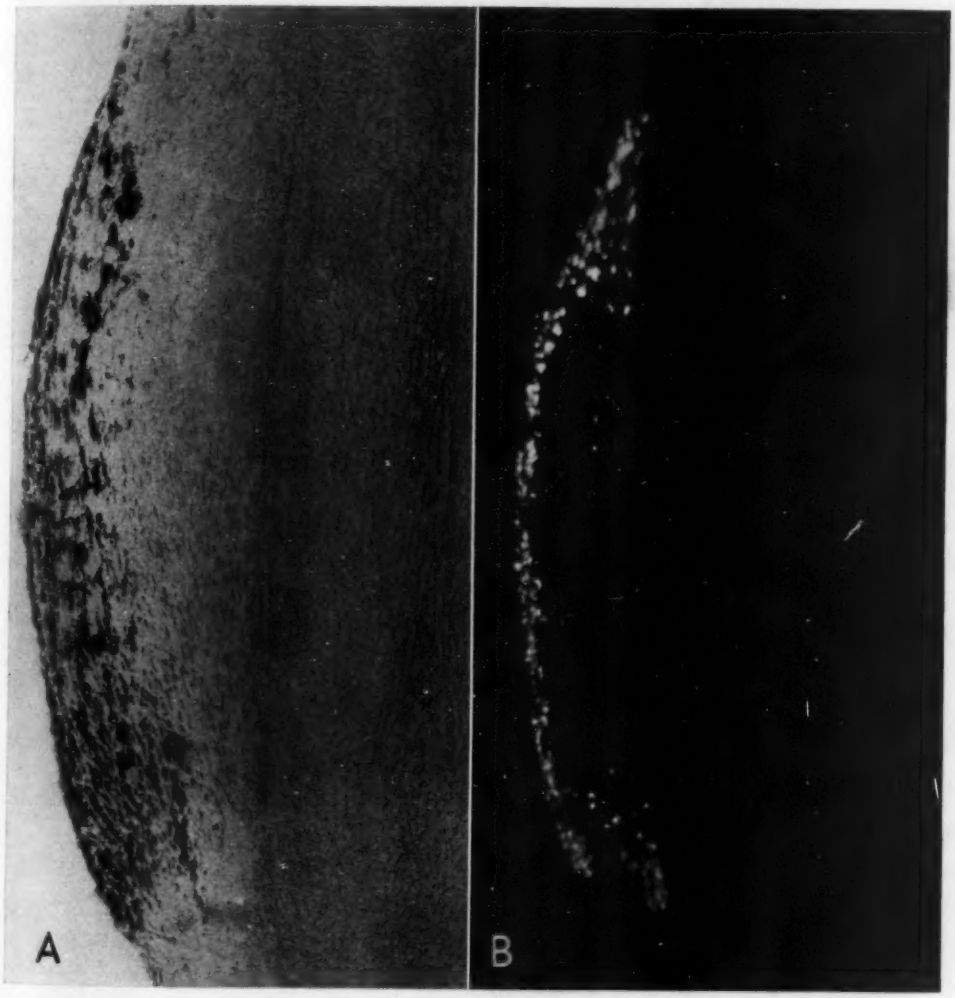

Fig. 9.—*A*, a nodule from the transverse arch. Globular lipophages are seen near the surface. Fibrolipophages below are lying in collagenous connective tissue. Necrosis is present, including the surface layer of media. The patient was 36 years old. Frozen section stained with hematoxylin and sudan IV; × 133. *B*, polariscopic photograph of an unstained frozen section near the one shown in figure 16; × 33.

polariscopic photograph of this field shows that the necrotic material in the base contains myriad minute crystals of cholesterol (fig. 10 *B*).

Figure 11 *A*, a section of a whiter nodule, shows more extensive formation of scar tissue, with a smaller central cavity filled with necrotic material. In figure 11 *B* is seen a section of the surface layer of a somewhat more advanced white scarred nodule. Introduction of lipoid from the surface has ceased. The layer directly under the epithelium is made up of scar tissue with no lipoid contents. Subjacent to this is a layer of scar tissue in which lie elongated cells of the fibrolipophage

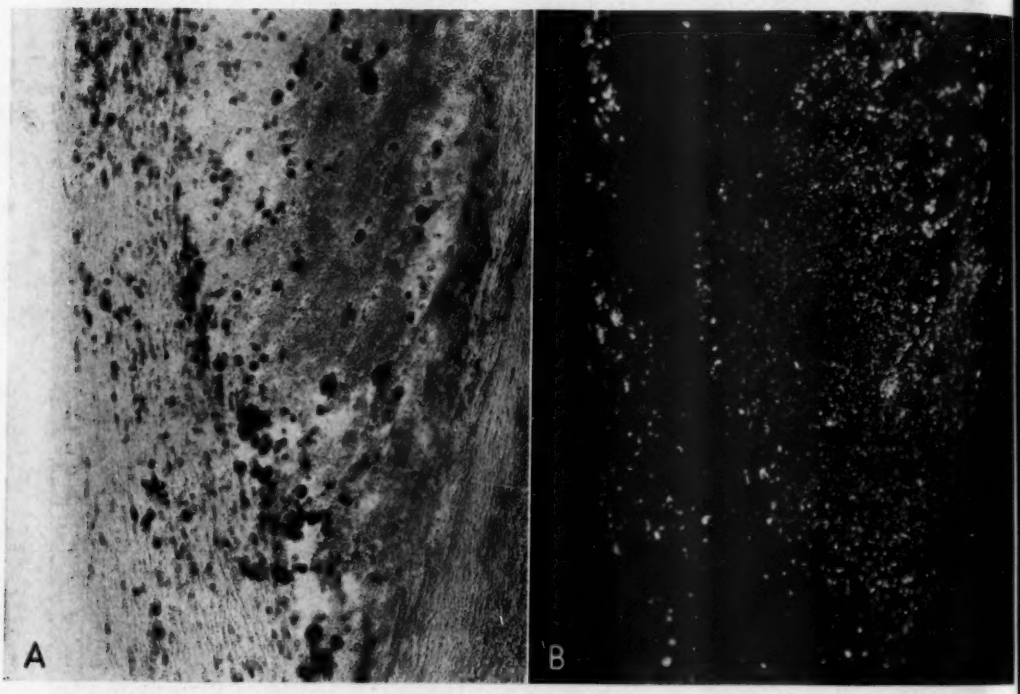

Fig. 10.—*A*, portion of an aortic lesion. The patient was a man aged 46. Globular lipoid cells and scar formation are present, and deep necrosis is noted below. Frozen section stained with hematoxylin and sudan IV; × 53. *B*, polariscopic photograph of an unstained frozen section near the one shown in *A*; × 53.

type containing lipoid. Trapped in scar tissue, their metabolic activities must be handicapped. More deeply occur globular lipophages compressed and elongated by the pressure of the scar tissue. Figure 12 *A* is from a field in such a lesion as is shown in figure 11 *A*, midway between the scar tissue at the surface and the necrotic mass below. Bands of scar tissue separate rows of globular lipophages. Figure 12 *B* is a polariscopic photograph of a scarred lesion with central

necrosis in the depths. Very few cholesterol crystals are seen at or near the surface. The rows of globular lipophages in the mid-region are seen as brilliant white bands. The cholesterol in the necrotic mass in the depths of the lesion is apparently present in fused discrete crystalline masses, unlike the picture seen in figure 10 *B*, in which the lipoid is present in minute crystals.

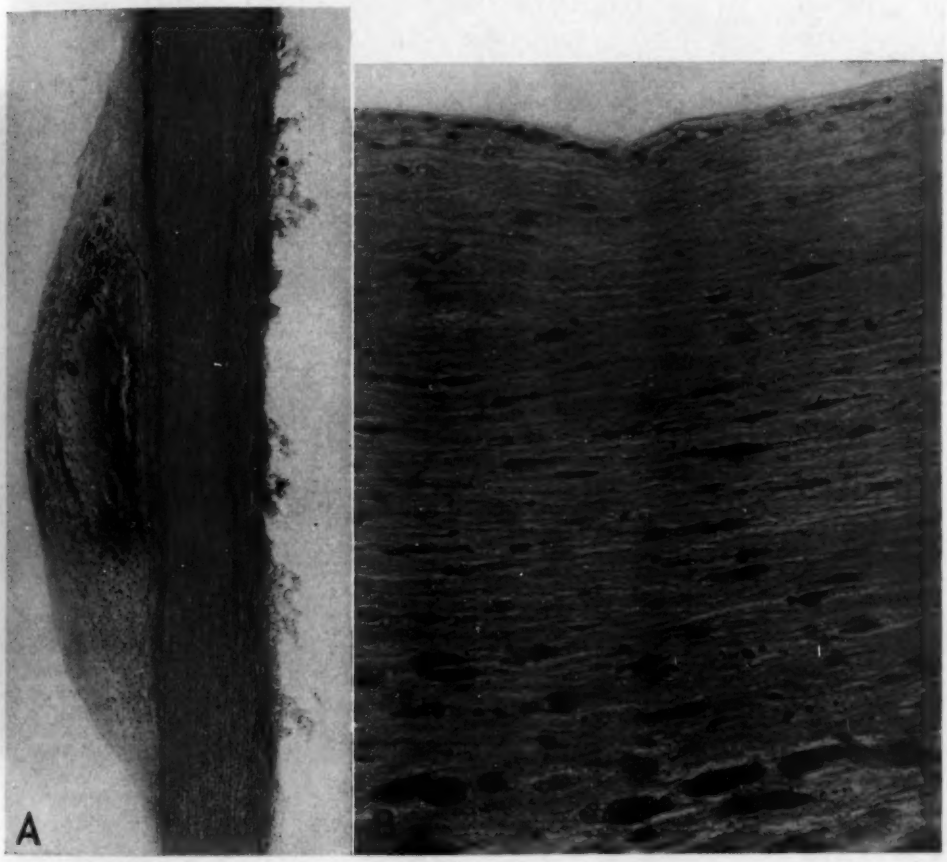

Fig. 11.—*A*, section of scarred yellowish nodule; × 10. The patient was 52 years old. *B*, detail of the surface of a scarred lesion; × 218. No globular lipophages are seen at the surface. Scar tissue is located near the surface. Few globular lipophages are present below. The patient was 50 years old. Frozen sections stained with hematoxylin and sudan IV.

Repair may occur, with the formation of scars which are silvery white on the surface and which show no yellow color on gross section. Striking examples are seen about intercostal orifices (fig. 13 *A*). Even in such white scars, globular lipophages may be present in considerable numbers (13 *B*).

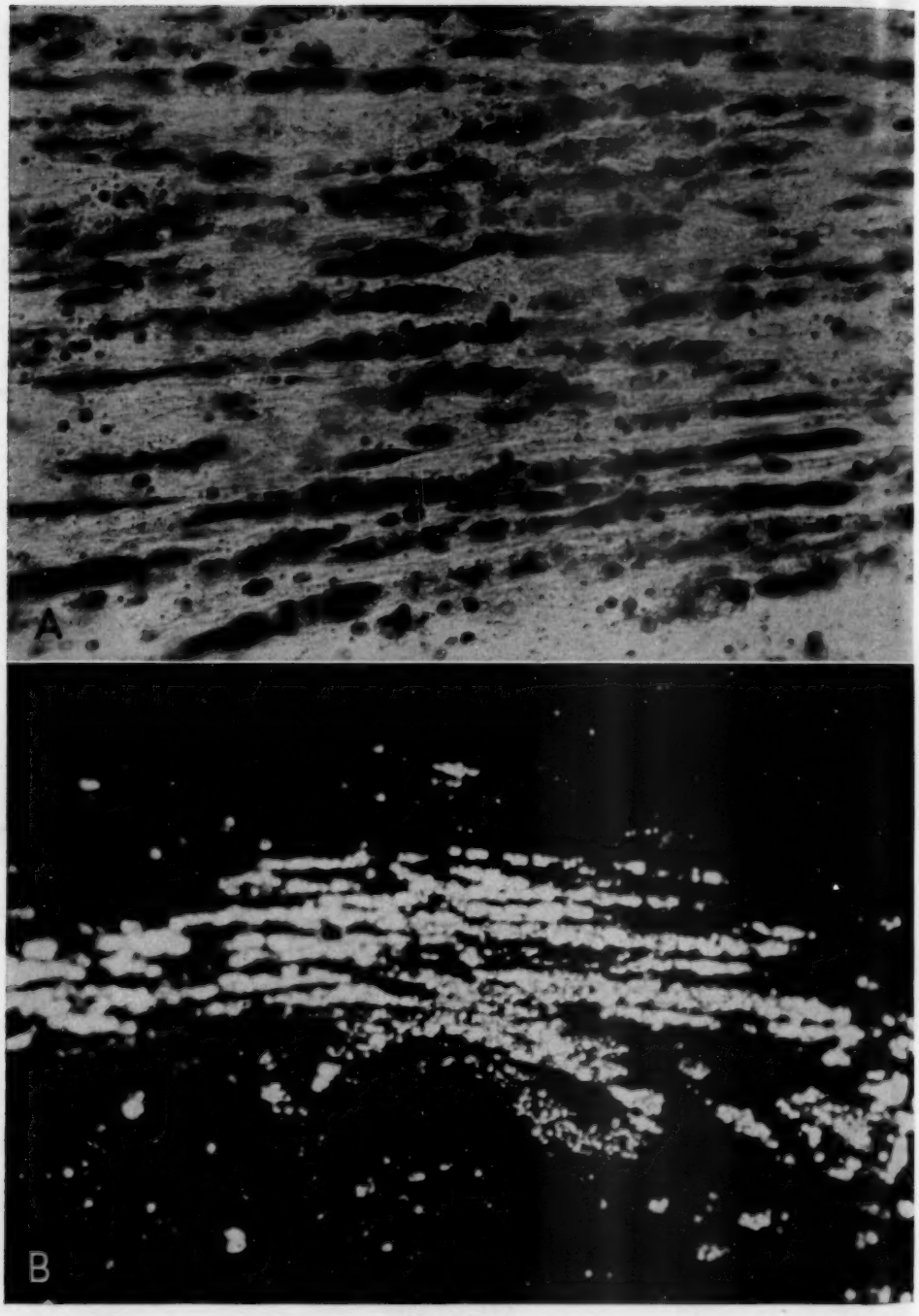

Fig. 12.—*A*, midregion of the lesion shown in figure 11 *B*. Rows of globular lipophages lie between bands of scar tissue. Frozen section stained with hematoxylin and sudan IV; × 312. *B*, polariscopic photograph of a portion of the lesion shown in figures 11 *B* and 12 *A*. Little cholesterol is seen near the surface; masses are present in the globular lipophages in the midregion; isolated masses are seen in the necrotic base. Frozen section; unstained; × 110.

III. Old Age: In old age the power to metabolize cholesterol, which has grown progressively weaker during the middle period of life, is essentially lost. Globular lipophages alone are present. Fibro-lipophages are not formed. The lipophages accumulate in masses under

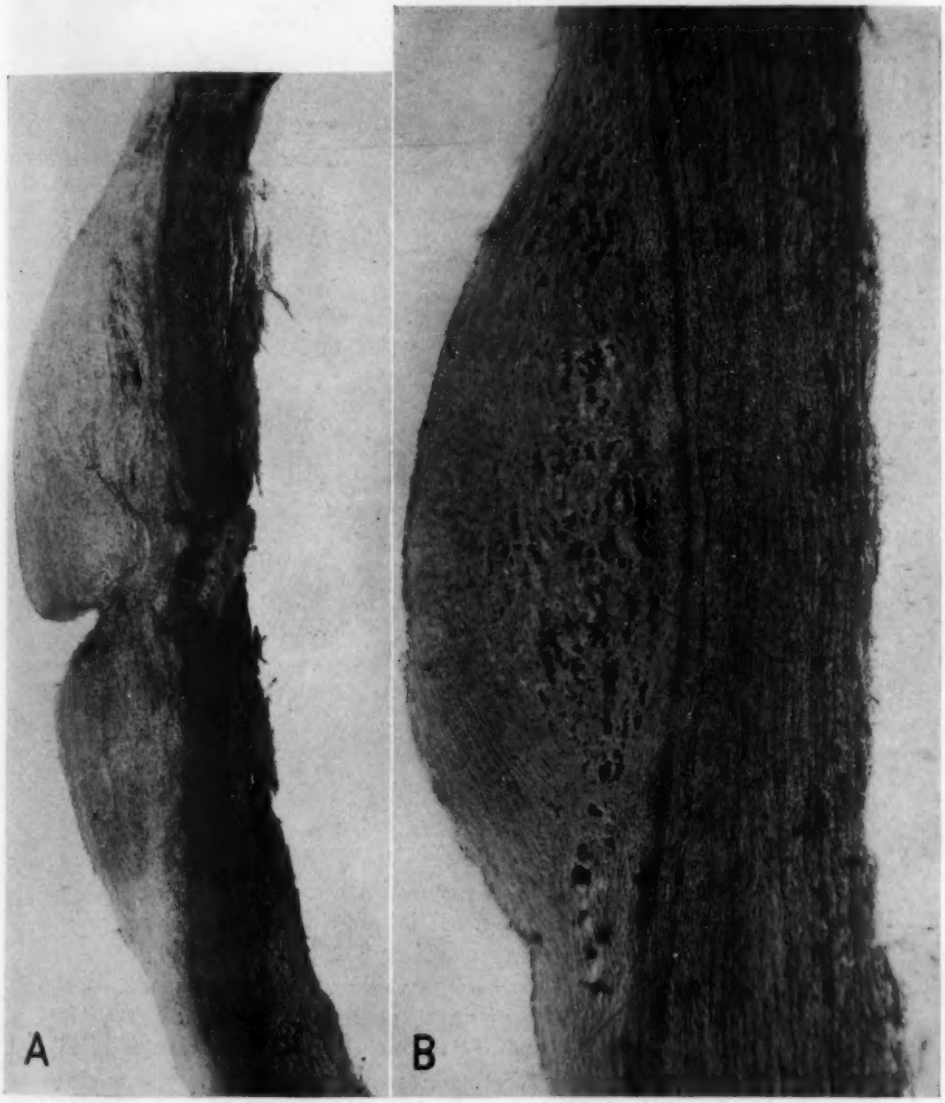

Fig. 13.—*A*, healed scar about an intercostal orifice; × 12. Note the vasa vasorum in the scar above the midline. The patient was 56 years old. *B*, apparently healed white scar with groups of globular lipophages; × 30. The patient was 52 years old. Frozen sections stained with hematoxylin and sudan IV.

the endothelium, with a minimal formation of connective tissue. No blood vessels are produced. Rarely a few branches of vasa vasorum may be present in the coronary lesions in this period, but I have never seen them in aortic lesions, except in lesions at the orifices of branches. The huge collections of cells suffer from lack of nutrition and lack of physical support. As a result necrosis occurs. This is rarely rapid and massive. Usually a slow necrobiosis begins in the middle of the cell mass and spreads gradually. There results the formation of a central cavity, the so-called atheromatous abscess (fig. 14 *A*). Necrosis of the cells is associated with a breaking down of the cholesterol esters, freeing cholesterol in solid crystal form. The crystals usually fuse into flat masses arranged in angular relation to one another in a mass of amorphous necrotic detritus. Most of these cavities are lined with globular lipophages. The innermost layers of these cells next to the cavity show vacuolation, with fusion of the droplets of their lipoid contents, and free cells undergoing necrosis but still identifiable are present in the necrotic detritus (fig. 14 *B*). In connection with long continued atheromatous "abscesses" a gradual necrosis and fibrosis of the media may lead to marked thinning of this layer (fig. 15 *A*). In some cases the lesion may extend through the media and may give rise to definite projection of an "abscess" through the media as a thin-walled sac covered only by adventitia. Most atheromatous "abscesses" are relatively massive and are not limited to the confines of single microscopic fields. The illustrations of these lesions have been selected perforce from small foci. Atheromatous "abscesses" may be associated in old lesions with fibrosis of the underlying media and are commonly the site of calcification. Frozen sections of these lesions are unsatisfactory because the thin covering layer of living cells tends to rupture, freeing the mushlike contents.

Rupture of these foci into the aorta may occur during life. The semisolid contents are mixed with the aortic blood stream, which breaks the mass up into small units which are capable of plugging capillaries and are perhaps responsible for some of the multiple cortical scars which may be seen in the kidneys. It is only in smaller arteries with narrowed lumens, e. g., coronary arteries, that the semisolid contents remain coherent and can produce obstruction at the point of rupture. If the tear in the inner layer of an aortic atheromatous "abscess" is small, the cavity produced by the delivery of the contents becomes filled with blood which clots (fig. 15 *B*). After a time this undergoes disintegration, with the deposit of pigment, which may persist for a long time. If the surface layer is torn off, exposing a large area, a more or less permanent ulceration may result, and thrombosis may follow. Rupture of an atheromatous "abscess" in the opposite direction is a rare condition. I have seen an enormous atheromatous "abscess"

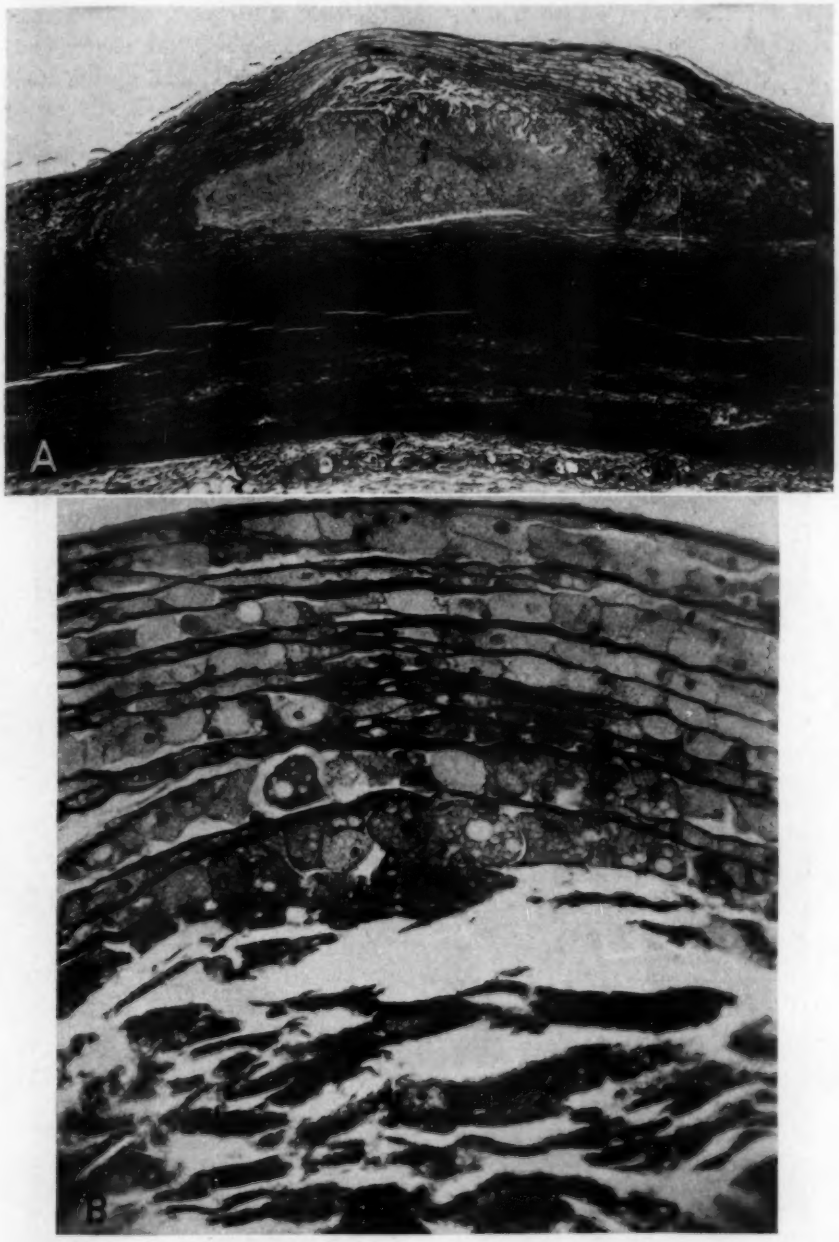

Fig. 14.—*A*, primary atheromatous "abscess;" × 24. The patient was 51 years old. *B*, detail (× 230) of the surface layer of the primary atheromatous "abscess" shown in *A*. Section fixed in Zenker's fluid and embedded in paraffin, stained with Mallory's aniline blue connective tissue stain.

extending from just below the orifices of the renal arteries down into the left iliac artery and including the whole circumference of the aorta. Rupture of the thin inner layer, with hemorrhage into the cavity and then through the outer covering made up of the thinned media and

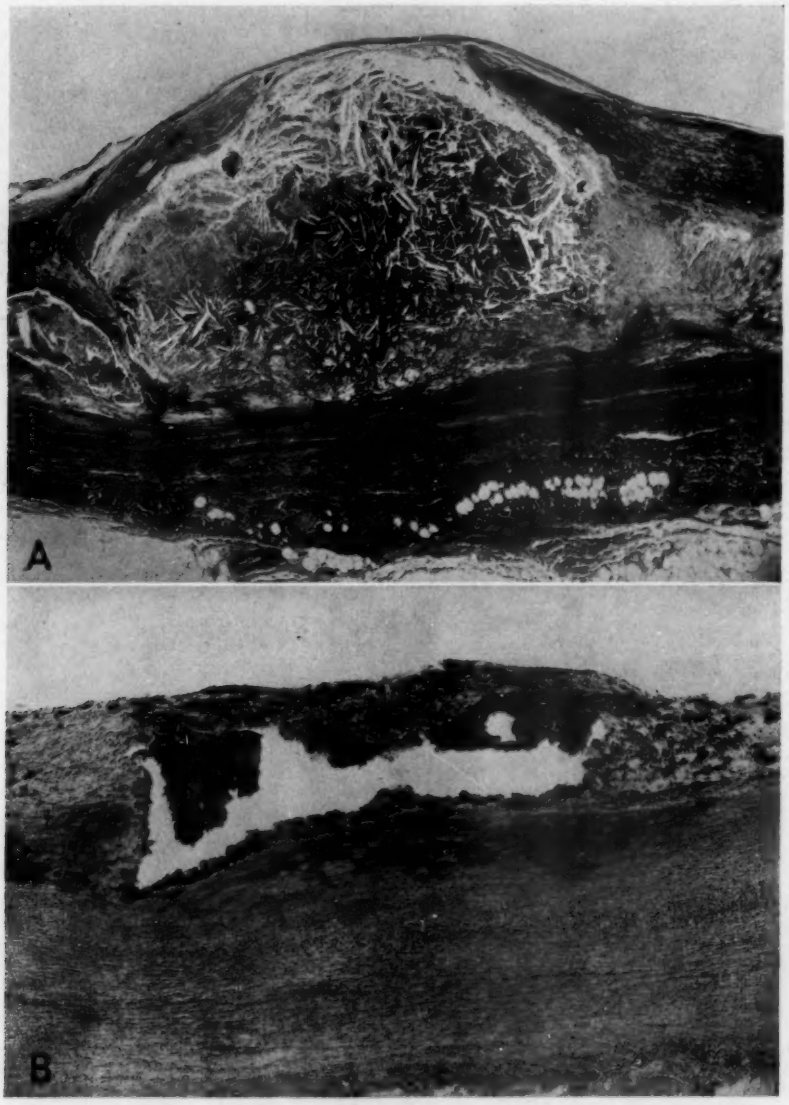

Fig. 15.—*A*, old atheromatous "abscess," with marked thinning of the media. The patient was 58 years old. Section fixed in Zenker's fluid and embedded in paraffin, stained with Mallory's aniline blue connective tissue stain; × 20. *B*, ruptured atheromatous "abscess." The cavity contains blood clot. The patient was 44 (?) years old. Frozen section stained with hematoxylin and sudan IV; × 40.

the adventitia gave rise to fatal hemorrhage into the retroperitoneal tissues.

Atheromatous "abscesses" occasionally occur in younger persons, in whom a combination of ineffective cholesterol metabolism and excessive cholesterol in the diet may result in studding the aortic lining with a series of these "abscesses" instead of the usual lesions described.

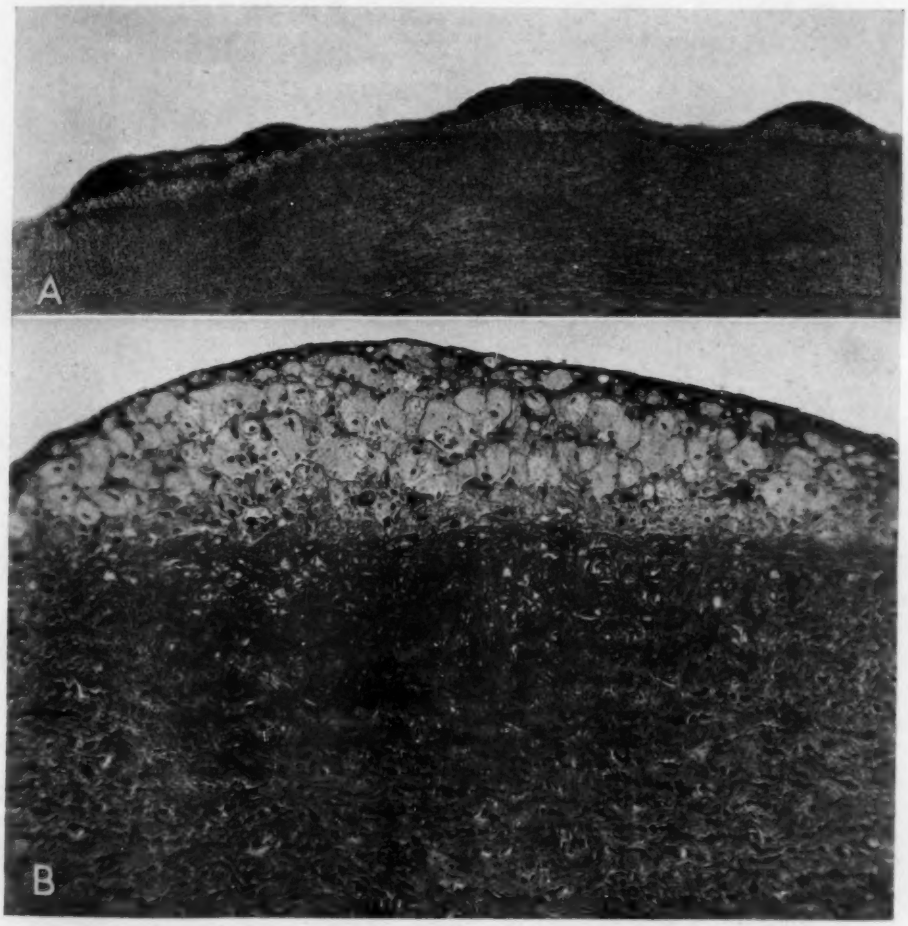

Fig. 16.—*A*, characteristic pinhead nodules of the ascending arch. The patient was 42 years old. Frozen section stained with hematoxylin and sudan IV; × 18. *B*, detail of a pinhead nodule of the ascending arch. Note the necrosis of the surface layer of the media. The patient was 32 years old. Section fixed in Zenker's fluid and embedded in paraffin, stained with Mallory's aniline blue connective tissue stain; × 150.

In the deep layers of advanced lesions, in which no evident lipoid cells persist, a form of sudan-staining fat is commonly seen. This fat

is not anisotropic and appears as fine droplets distributed in wavelike masses or diffusely through the necrotic base layer, but without the formation of a definite cavity. It is apparently due to secondary fatty infiltration of the necrotic material (fig. 19*B*). It may include the surface layers of the media or may appear in patches in the media

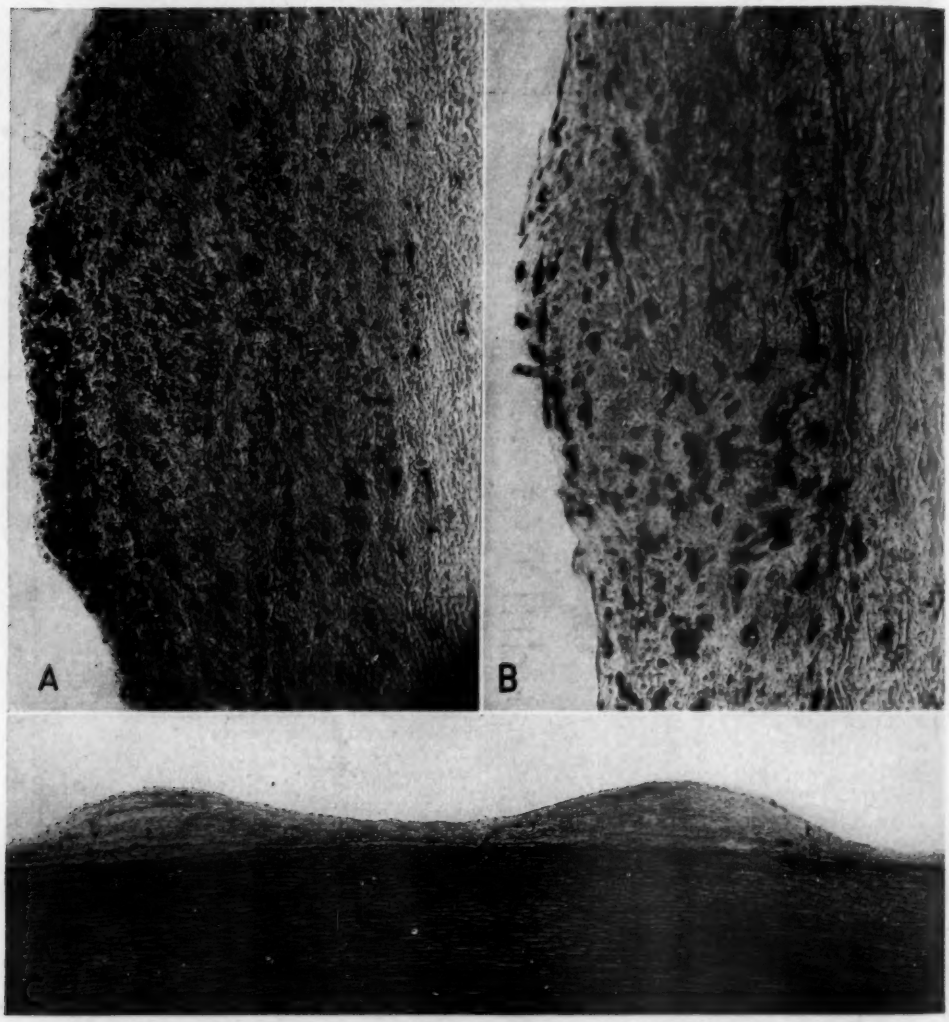

Fig. 17.—*A*, section of a pinhead nodule of the ascending arch; × 110. Note the fat in the outer layers of the necrotic media. The patient was 33 years old. *B*, fibrolipophages in pinhead lesion of the ascending arch; × 23. The patient was 65 years old. *C*, healing pinhead nodules of the ascending arch, showing loose reticular tissue; × 200. The patient was 69 years old. Frozen sections stained with hematoxylin and sudan IV.

beneath the lesion, apparently unconnected with the intimal process (fig. 15 *B*).

IV. Exception: Lesions of the ascending arch of the aorta do not follow the rules just indicated. The ascending arch sustains the highest pressures to which the aortic wall is subjected. It has no branches, however. Yellow opaque deposits along the aortic ring are common. The ascending arch, while it may show greater aggregations of unit lesions than any other part of the vessel, is not commonly the site of advanced or large unit lesions. The common picture is that of a collection or collections of pinhead-sized yellow foci (fig. 16 *A*). These are bright yellow mounds when fresh but paler gray as they age. The bright yellow mounds are made up of closely packed collections of globular lipophages. These lesions, unlike most of the lesions described, tend to be accompanied with necrosis of the inner layer of the media (fig. 16 *B*), and deposits of lipoid are present not only in the globular lipophages beneath the endothelium but also in the necrotic media (fig. 17 *A*). As the lesion ages there is produced a reticular connective tissue the fibroblasts of which are lipophages and metabolize the cholesterol without reference to the age of the subject (fig. 17 *B*). When the introduction of fresh cholesterol ceases, the metabolism within the fibrolipophages removes the lipoid efficiently even in persons of advanced ages. When the lipoid has been completely removed, there remain small mounds of delicate reticular connective tissue (fig. 17 *C*). These ultimately sink back into the surface layer and may appear as small gray spots without projection.

The explanation of the peculiar lesions in the ascending arch appears to be that this part of the vessel swings free and its expansion and contraction occur in continuous waves, uninterrupted by the breaks occurring at the orifices of branches. When this freedom of muscular action is interfered with, as in syphilitic aortitis, massive atherosclerotic lesions frequently appear, ingrafted on the basic syphilitic process. The ability of fibrolipophages to metabolize cholesterol in this location in persons of advanced age seems to depend on the laying down of a loose-textured reticular connective tissue with little or no formation of collagen, i. e., young fibroblastic tissue. The constant stretching may prevent the formation of scar tissue. Successful metabolism of cholesterol and its removal are met with in the ascending arch relatively late in life, when all other parts of the vessel show lesions evidencing more or less complete loss of the metabolic power. Moreover, the reticular connective tissue gives rise to minimal scarring, so that this part of the vessel may present a smooth surface, though often with pale gray spots or lines, while the rest of the vessel shows extensive advanced atherosclerotic lesions.

CALCIFICATION

Calcification occurs in atherosclerotic lesions in two forms. The common aortic process is associated with fatty changes in tissues undergoing necrobiosis. The deposits of calcium seem to occur first about the nuclei of dying cells. Drops of lipoid and calcified granules are present within the same cell remains (fig. 18*A*). In other lesions, particularly those arising from rapid mass necrosis, the calcium may appear

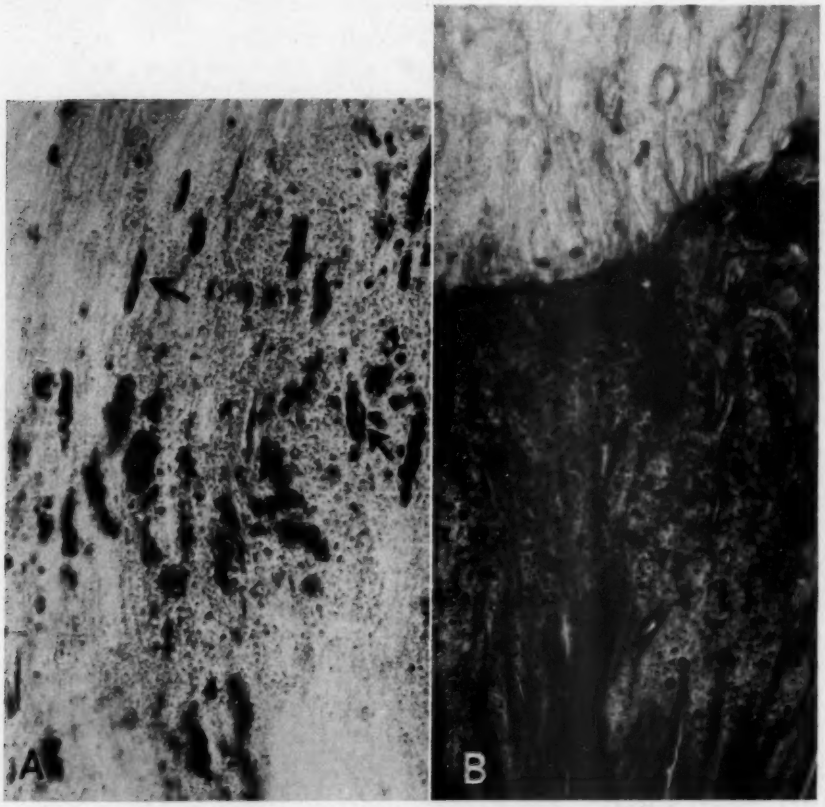

Fig. 18.—*A*, early calcification in an aortic lesion undergoing necrobiosis. Fat and calcium nodules are present in the same cell remains. The lighter-colored regions in the cell remains, as indicated by arrows, are fat stained with sudan. The darker masses are calcium stained with hematoxylin. Frozen section stained with hematoxylin and sudan IV; × 385. *B*, coagulation necrosis with calcification. Note the drops of material containing calcium. Section fixed in Zenker's fluid and embedded in paraffin, stained with Mallory's aniline blue connective tissue stain; × 285.

in fine granules which tend to fuse (fig. 18*B*). Fatty changes are less important in this form. As the calcium increases in amount, it tends

to form plaques the surface layer of which may be covered by a layer of endothelium with a minimal amount of connective tissue (fig. 19 *A*). Calcification is always a terminal process, a monumental deposit in dead or dying tissues.

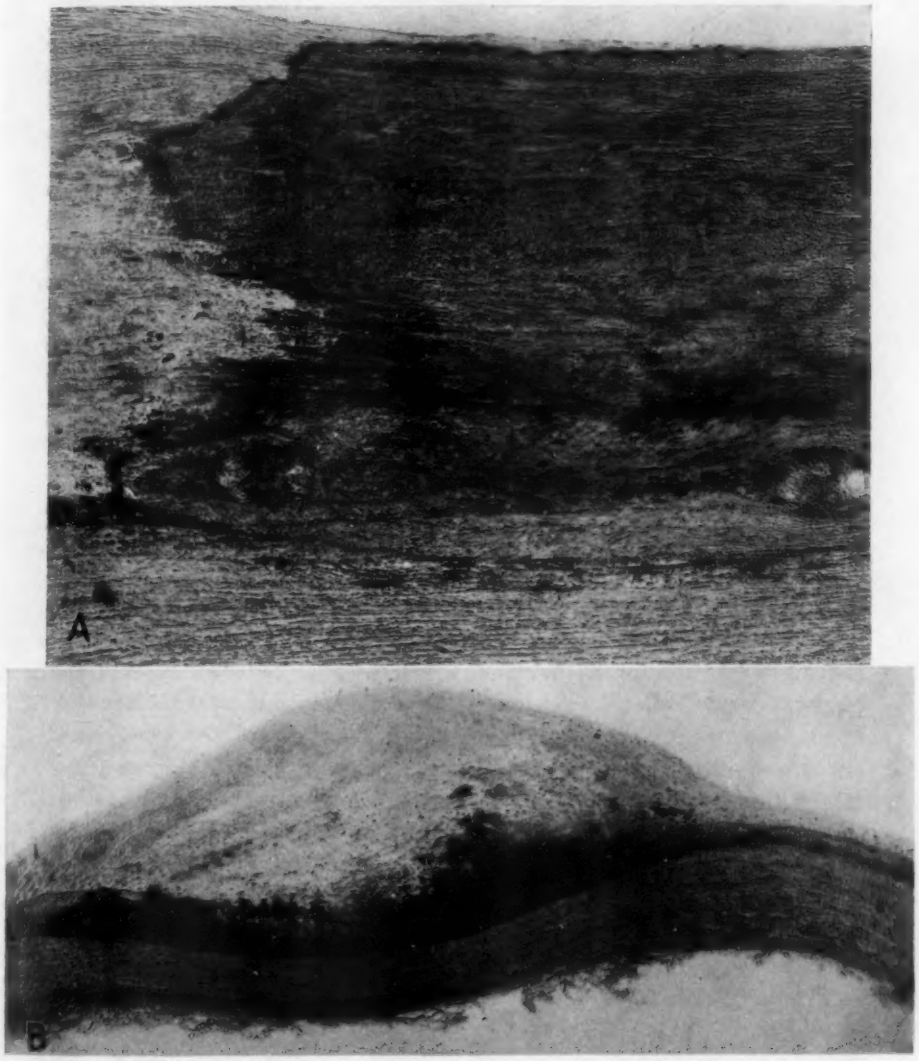

Fig. 19.—*A*, calcified plaque beneath the endothelium. The oval structures in the lower left and right areas are encapsulated masses of fused cholesterol crystals. Section fixed in Zenker's fluid and embedded in paraffin, stained with Mallory's aniline blue connective tissue stain; × 57. *B*, scarred aortic nodule showing necrosis and secondary fatty metamorphosis in the base. Frozen section stained with hematoxylin and sudan IV; × 15.

Variety in the aortic lesions as seen grossly is greatest in the late middle period. Scarred and calcified lesions tend to persist during life. Examination of the aorta of an old person will perhaps reveal yellow deposits and some plaques of lime salts about the ring; a mingling of small recent lesions the size of a pinhead in the ascending arch with minimal scars from old processes visible as gray spots or streaks and sometimes including a vertical scar along the region of contact of the aorta and the pulmonary artery; calcified lesions with scarring about the orifices of the branches of the aortic arch; a calcified scarred lesion at the site of the orifice of the ductus arteriosus, groups of scars about the intercostal branches with some atheromatous "abscesses," yellow spots and streaks and older, partly scarred mounds on the anterior wall of the thoracic vessel; scarring with some plaques of lime salts about the orifices of the larger abdominal branches and a mixture of scars, plaques, ruptured and unruptured atheromatous "abscesses" and yellow areas often covering the whole surface of the lower portion of the abdominal vessel. The dominant lesions in youth are yellow spots and bands; in the early middle period, scars; later, atheromatous "abscesses" and calcified plaques.

EXPERIMENTAL ATHEROSCLEROSIS IN THE RABBIT

Study of the evolution of aortic lesions [1] in the cholesterol-fed rabbit discloses essentially the same phenomena that I have described as occurring in human beings. The mutations observed in the experimental disease correspond, particularly in the early stages, with those appearing in lesions in human beings. These include the mucoid swelling of the subendothelial layer of the intima, the infiltration with isotropic lipoid, the appearance of globular lipophages containing anisotropic lipoid, the diffuse increase of reticular connective tissue, the occurrence of fibrolipophages and the evident metabolism of the lipoid. The excessive doses of cholesterol lead to rapid production of massive processes which progress long after feeding has ceased. The youth of the animals permits of efforts at metabolism of the cholesterol in fibrolipophages in the surface layers of far advanced lesions. There appears to be a tolerance to the presence of cholesterol crystals associated with fibrosis in the depths of old processes which is not manifest in the reactions in human beings. In the rabbit a massive layer of dense scar tissue may cover the surface, and yet the tissues in the depths may exhibit little or no necrosis in spite of large collections of cholesterol crystals. The production of frank atheromatous "abscesses" does not, therefore, ordinarily occur in the rabbit, though I have produced this lesion in smaller arteries. This difference is again probably the result of youth and is related apparently to better nutrition of the depths of thick lesions than is usually present in similar lesions in human beings.

Collagen formation with fibrosis occurs relatively earlier in the rabbit than in man. This is due perhaps to the impossibility of adequate cholesterol metabolism and removal in the face of the continuous large doses of the substance and a resulting delay in repair. In human beings the delay in the repair by reticular tissue may lead to collagen formation and scarring even in youth.

My observations have been limited, as indicated, to animals which lived for a maximum of nine months after cessation of the feeding of cholesterol. Anitschkow's [3] description of the lesions in animals which were permitted to live for two or three years after cholesterol feeding had been discontinued demonstrated that progressive repair with fibrosis is associated with complete disappearance of the lipoid and healing by scar formation, sometimes with calcification.

Though the disease in rabbits and that in human beings progress along parallel lines, a greater diversity of stages is available for study in human beings from birth to old age than is readily obtained under experimental conditions. For this reason and because of the greater practical importance of the disease in human beings, attention has been focused on the human process in this paper. It should be stressed, however, that with due allowance for time, age, dosage of cholesterol and difference in species, generically the processes correspond in animals and man.

THE STIMULUS TO GROWTH OF CONNECTIVE TISSUE

The body as a whole presents many examples, particularly in the organization of dying cellular exudates, of the stimulation of the growth of connective tissue by free solid cholesterol crystals. In the ester form the substance is more bland. As has been seen in early lesions in the aorta in old age, the lipoid remains in the ester form in globular lipophages and does not undergo metabolic changes. There is an associated lack of formation of connective tissue. In youth, when the metabolism of lipoid is most active and rapid, connective tissue is formed. The only cholesterol in solid crystals is in the minute crystals in fibrolipophages, but collagen formation does not occur. In the middle period of life, metabolism is slowed, connective tissue is formed, again in the absence of cholesterol in solid crystals save in the few fibrolipophages, but collagen formation results in the production of scar tissue.

The suggestion is that some soluble product of cholesterol metabolism other than free solid cholesterol crystals is the agent responsible for the stimulation of the diffuse growth of connective tissue in atherosclerotic lesions in youth. In connection with atheromatous "abscesses"

3. Anitschkow, in Cowdry, Edmund V.: Arteriosclerosis: A Review of the Problem, New York, The Macmillan Company, 1933.

free solid crystals are present and may stimulate the growth of connective tissue in the walls. This stimulation is, nevertheless, relatively unimportant, since the crystalline cholesterol tends to be embedded in the necrotic detritus within the cavity of the atheromatous "abscess," and its contact with living connective tissue cells is limited. However, in relation to very old atheromatous "abscesses," marked scar formation, which often includes the neighboring media, may occur.

THE LIPOPHAGES

It is generally agreed that phagocytosis is a function of relatively primitive types of cells which persist as such in the complex tissues even of the higher animals and retain the power of intracellular digestion.

The present problem is concerned with the cells active in the removal from the tissues of an inanimate substance, a lipoid. The literature is rich with reports of the study of phagocytosis of bacteria and of many forms of inanimate foreign substances by motile cells.

Only recently have extensive studies been carried out of the methods by which lipoids are taken into cells. This work has been reviewed by Kimmelstiel and Laas,[4] who succeeded by the intravenous injection of cerebroside into rabbits in reproducing morphologically the lesions ot Gaucher's disease, and who stated the belief that the process of entrance of lipoids into cells is not a true phagocytosis but a physicochemical procedure, depending on the formation of colloidal suspensions (emulsions) of the lipoids which are taken into the cell in extremely finely divided form. They indicated that the cell which takes up the lipoid plays a more or less passive part, the ability of finely divided colloidal suspensions to enter the cytoplasm through the mosaic structure of the surface of the cell being the dominating factor. If the cell is a passive agent it is reasonable to conclude that cell specificity plays no part in the process. The fact that the cells which first take up these lipoids are always the standard macrophages signifies a specific selective activity on the part of these cells. The popular belief that particulate matter is engulfed by phagocytes only through the formation of relatively gross pseudopods, which surround a foreign particle and thus introduce it into the cell body, has been disproved repeatedly in studies of the phagocytosis of bacteria. The single bacterial body, after lying in contact with the surface of the cell, is incorporated in the cell body by a process which does not include the formation of demonstrable pseudopods. This is true also of finely divided particles of inanimate matter. The taking of particulate foreign material into the cell body is phagocytosis, regardless of the size of the particles.

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4. Kimmelstiel, P., and Laas, E.: Beitr. z. path. Anat. u. z. allg. Path. **93**: 417, 1934.

A study of frozen sections of aortic lesions suggests that a simple explanation of the phenomena of phagocytosis of cholesterol is that the globular lipoid cells which first ingested the substance took on vigorous ameboid activity and were converted into the branching phagocytes as the lesions progressed in youth and as subendothelial reticular tissue was produced.

The demonstration by special stains that the branching phagocyte is a fixed cell, a fibroblast, markedly complicates the issue.

The present problem deals with the question whether the two distinct forms of phagocytic cells observed represent two phases in the life of a single cell type or two distinct cell types.

In favor of a belief that these may represent phases in the evolution of a single cell was the early observation of Metchnikoff,[5] who said:

Dans mon premier travail sur l'inflammation des amphibies (Biol. Centralbl., 1883) j'ai insisté sur le fait que les cellules fixes étoilées du tissu conjonctif présentent également des propriétés phagocytaires. On peut facilement observer sur des nageoires enflammées depuis pleusieurs jours des cellules munies de prolongements ressemblant à des bois de cerf, par conséquent des éléments fixes caractéristiques, renfermant dans leur protoplasma des corps étrangers, comme des grains de carmin ou des débris de globules rouges. De ces faits, que j'ai pu confirmer à plusieurs reprises, j'ai conclu que les cellules fixes étaient des phagocytes correspondant aux cellules migratrices. Eh bien, j'ai reconnu depuis que cette interprétation était inexacte. Malgré des tentatives nombreuses, je n'ai jamais réussi à constater l'englobement des corps étrangers par les prolongements protoplasmiques des cellules fixes. Les recherches dirigées vers ce point, et répétées pendant plusieurs années de suite, m'ont persuadé que les cellules fixes définitivement formées ne s'incorporent jamais des grains de carmin ou autres corps étrangers. Ceux qui se trouvent dans leur protoplasma ont été englobées dans un état antérieur de dévelopment, lorsque les cellules étaient encore phagocytes mobiles. Ces faits nous fournissent donc une preuve certaine du passage des cellules migratrices à l'état d'éléments fixes. Quoique ce résultat se trouve en desaccord avec l'opinion presques unanime des pathologistes, il est néamoins parfaitement réel.

(In my first work on inflammation in amphibia I insisted on the fact that the fixed star cells of the connective tissue also exhibit phagocytic properties. In swimming appendages inflamed for several days one can readily observe cells armed with processes resembling stag-horns, therefore characteristic fixed elements, enclosing in their protoplasm foreign bodies such as grains of carmine or the débris of red blood cells. From these facts, which I confirmed on several occasions, I concluded that fixed cells were phagocytes corresponding to wandering cells. Since then I have come to know that this interpretation was inexact. In spite of numerous attempts I have never succeeded in establishing the englobement of foreign bodies by the protoplasmic processes of fixed cells. Researches with this in view and repeated during several successive years have persuaded me that definitely formed fixed cells never incorporate grains of carmine or other foreign bodies. Those which occur in their protoplasm have been absorbed during a

5. Metchnikoff, E.: Leçons sur la pathologie comparée de l'inflammation, Paris, G. Masson, 1892, p. 124.

previous stage of development when these cells were still mobile phagocytes. These facts then furnish definite proof of the passage of wandering cells into the state of fixed cells. Although this result is in disagreement with the almost unanimous opinion of pathologists, it is nevertheless absolutely true.)

Pathologists, however, remain obdurate in their disbelief that motile cells can be converted into fibroblasts. The elongated cells resembling fibroblasts and containing indigestible pigment that are seen in lymph nodes and scar tissue are regarded as macrophages which have migrated and come to rest there.

The concept which deals with the possible duality of the cells active in the removal of cholesterol is looked on as revolutionary, since it confers on fixed cells—fibroblasts—the power of phagocytosis. The natural reaction of the pathologist is to suggest that fatty material present in fibroblasts is there as the result of degeneration of the cell.

The evidence from studies of aortic lesions is as follows:

The lipophage of the monocyte-histiocyte type—the globular lipophage—is palpably incapable of adequately metabolizing cholesterol. In old age globular lipophages are present in the lesions in great numbers. No fibroblastic or other cells containing lipoid are present. The lipoid remains within the globular cells in the form of cholesterol esters—unchanged—until necrosis of the cells sets free the esters, which are then split, depositing free cholesterol in excess in the necrotic detritus, where it remains indefinitely—also unchanged—in the contents of atheromatous "abscesses." Frequently this cell type tends to show evidence of necrobiosis even in the lesions of the young.

The fibrolipophages, per contra, are associated with tangible evidence of the metabolism and removal of cholesterol from the lesions. The demonstration that these cells are fibroblasts is beyond question, since they form fibroglia fibrils. They are seen almost exclusively in the lesions of young subjects. They arise later in the lesions than the globular lipophages and persist after the globular lipophages have disappeared. The occurrence of the lipoid within these cells is unaccompanied with any of the stigmas of degeneration. The appearance of the cells containing lipoid suggests function rather than degeneration. The fat of degeneration is isotropic; the lipoid in these cells is anisotropic, i. e., cholesterol, before it is metabolized. These cells seem to be particularly hardy, and morphologic evidences of degenerative changes in them are exceedingly rare, in contrast to the common occurrence of degenerative changes in the globular lipophages. Essentially all the newly produced fibroblasts in a given region take on the phagocytosis of the lipoid. With its disappearance they revert to simple fibroblasts.

Smith [6] has pictured the intake and digestion of melanin granules in the fibroblasts of chick embryos in tissue cultures. Lewis,[7] studying the destruction of Bacillus radicicola in cultures of connective tissue of the chick embryo, was able to differentiate two types of cells, i. e., the typical ameboid macrophage and the fibroblast. The macrophages ingested the bacteria much more readily than did the fibroblasts. The destruction of engulfed B. radicicola was, however, much more rapid in fibroblasts than in the motile macrophages. He concluded that:

> The great number of organisms and other foreign bodies which the connective tissue cells are able to destroy in tissue cultures suggests that these cells may frequently play a part in phagocytosis, but because of the rapidity with which the ingested organism is destroyed it is seldom observed in the animal body.

The greater efficiency of the fibrolipophages in the removal of cholesterol in aortic lesions is perhaps significant in this connection.

That the fibroblastic cells which metabolize cholesterol are relatively primitive from the developmental standpoint is suggested by their minimal formation of collagen in the repair of lesions in youth. As these cells undergo full differentiation (into fibrocytes?), when middle age comes on, they tend to take on the functions of adult cells, including the formation of collagen. With this change they cease to contain the lipoid, the metabolism of which is halted.

Fortunately the answer to the controversial question of the relation of these cells is not vital to the present study. Investigation of the subject is being continued.

NUTRITION OF THE LESIONS

All the evidence indicates that the nutrition of the lesions of atherosclerosis is wholly by imbibition from the blood stream through the endothelium. In youth, when the new tissue is loose-textured and reticular, good nutrition may be evident throughout the formation of even massive lesions (fig. 7). With the formation of collagen and of scar tissue, nutrition to the deep layers of the lesions may be inadequate, and necrosis may result.

VASA VASORUM

There is no evidence of vasa vasorum in any of the lesions, save occasionally in the late stages of lesions about the orifices of branches (fig. 14 *B*). Also in advanced lesions there sometimes appears to be an increase of the vasa vasorum in the media, with satelite groups of lymphoid cells, but the vessels do not penetrate the lesions. Moreover, the necrosis of the deep layers of such lesions negatives any claim that they supply nutrition to the intimal process.

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6. Smith, D. T.: Bull. Johns Hopkins Hosp. **32**:240, 1921.
7. Lewis, M. R.: Bull. Johns Hopkins Hosp. **34**:223, 1923.

ATHEROMATOUS ABSCESS

Two types of so-called atheromatous "abscess" have been described: 1. The primary type of atheromatous necrosis, which is met with in old age particularly. In this lesion the necrosis begins in the midregion of masses of lipoid cells and spreads in every direction. 2. The secondary type of atheromatous necrosis, which occurs particularly in the base of the scarred lesions in middle age. The necrosis in this type is due to the distance from the source of nourishment, i. e., the blood within the vascular lumen, and to the relative impenetrability of the covering layer of scar tissue. The consistency of the contents of this type is greater than that of the primary type. Because of the scarred surface layer, rupture, which is common in the primary type, does not occur.

The term "abscess" as applied to this lesion arose from the grossly puriform appearance of the contents of some primary lesions. It is a misnomer, since the process is remarkably free from cellular exudative elements, and infection is barred, probably because of the presence of concentrated cholesterol in the contents. A single word to designate this lesion is a desideratum. The term atheroma has been loosely applied not only to atheromatous "abscesses" but to other atherosclerotic lesions, notably the small yellow nodules. The words cyst, cavity and space are not distinctive and need the qualification atheromatous. I suggest that the term atherocheuma, compounded of ἀθήρη and χεύμα (a melting or liquefaction), fills the requirements. The word is definite in meaning, its derivation is authorized,[8] and it is readily pronounced with the accent on the penult.

FACTORS WHICH INFLUENCE THE DEPOSIT OF CHOLESTEROL IN ARTERIES

(*a*) *Hypercholesteremia.*—In the experimental animal an excess of cholesterol in the diet is the important element. In cases of diabetes during the period when high fat diets were given, this was apparently true. In both instances a high cholesterol content of the blood is the rule. This is true also in myxedema, which tends to be accompanied with marked arteriosclerosis.

There is much debate as to whether a high cholesterol content of the blood is necessary for the production of atherosclerosis. In opposition to this belief is offered the evidence that in patients with advanced atherosclerosis the cholesterol content may not be high. Measurements of the cholesterol in the blood are of value in determining what the content is at the moment when the sample of blood is obtained. A few hours later the content may be markedly changed. Advanced athero-

8. By Dr. John H. Finley Jr., Harvard University.

sclerosis is the result of years of progressive accretion. To conclude that because the cholesterol content of the blood is low at a given moment after the disease is established, it was necessarily low during the active evolution of the disease is not good reasoning. In general it appears to be probable that, other predisposing factors being favorable, a high cholesterol content in the blood tends to facilitate the deposit of cholesterol esters in the intima and therefore the development of atherosclerosis.

(*b*) *Thyroid Secretion and Iodine.*—That a high cholesterol content of the blood is not the controlling factor in the experimental form is indicated by the studies of Page and Bernhard.[9] Rabbits fed the di-iodide of ricinsterolic acid in addition to cholesterol in oil showed a higher cholesterol content of the blood than control animals receiving only cholesterol in oil, but the degree of atherosclerosis produced in the animals receiving iodine was much less than that obtained in the cholesterol-fed controls. The empirical use of iodine in the treatment of atherosclerosis has been practiced for many years. In 1917 Murata and Kataoka[10] demonstrated that iodine could serve to protect rabbits against the deposit of dietary cholesterol in the arteries. This work has been confirmed by Liebig,[11] Turner[12] and Friedland.[13] Based on the observation that the cholesterol content of the blood tends to be low in subjects with hyperthyroidism and high in those with myxedema, Turner's experiments established the potency of the feeding of whole thyroid in checking the arterial deposit of dietary cholesterol and the inefficacy of iodine in this respect in thyroidectomized rabbits.

Mucoid Degeneration: Perhaps most important in this connection is the character of the change which the ground substance of the intimal connective tissue exhibits in early lesions. Aschoff referred to a molecular disintegration of the tissues, whatever that may mean. In a study of lesions of the coronary arteries in man, some of which were in very early stages, I was impressed by the appearance in paraffin sections of a mucoid change in the swollen intercellular substance of the connective tissue (fig. 20 *A*). Comparison of frozen sections stained for fat and paraffin sections from the same lesion disclosed that the intercellular substance exhibiting the mucoid change is the depositary of free lipoid. When the fat is extracted in preparation for paraffin embedding the mucoid tissue is left behind as a substratum.

9. Page, I. H., and Bernhard, W. G.: Arch. Path. **19**:530, 1935.
10. Murata, M., and Kataoka, S.: Verhandl. d. jap. path. Gesellsch. **7**:27, 1917.
11. Liebig, H.: Arch. f. exper. Path. u. Pharmakol. **159**:265, 1930; **175**:409, 1934.
12. Turner, K. B.: J. Exper. Med. **58**:115, 1933.
13. Friedland, I. B.: Ztschr. f. d. ges. exper. Med. **87**:683, 1933.

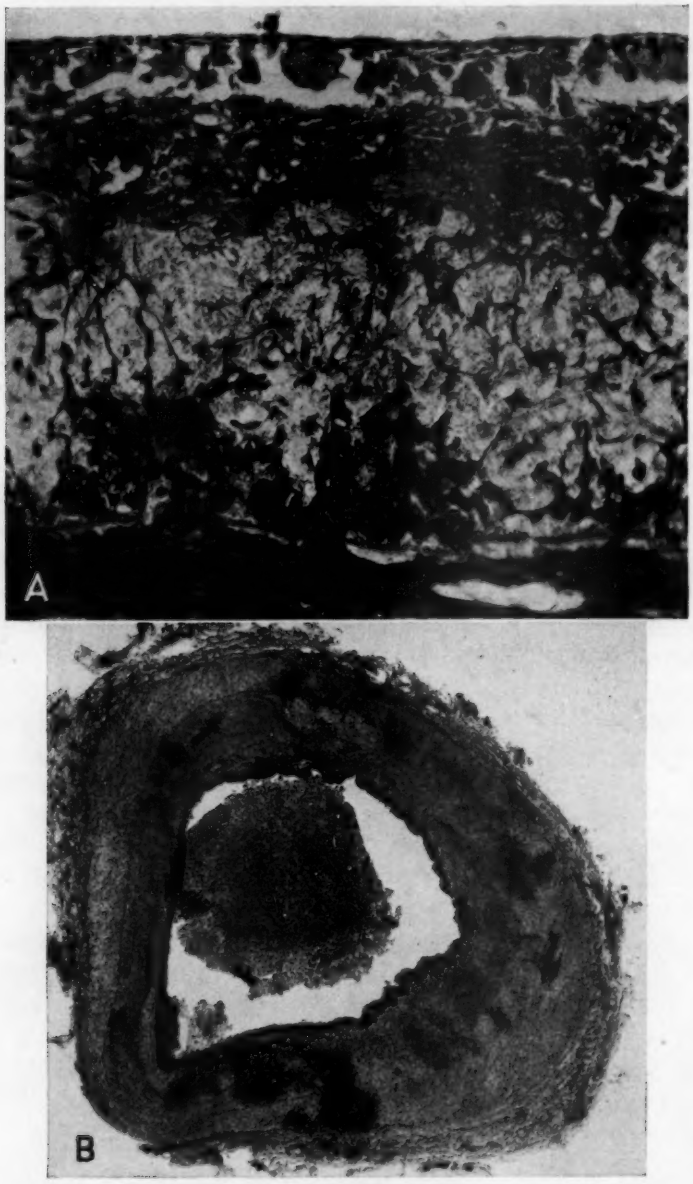

Fig. 20.—*A*, coronary sclerosis. Mucoid tissue is present in the subendothelial layer above the bundles of longitudinal muscle fibers. Section fixed in Zenker's fluid and embedded in paraffin, stained with Mallory's aniline blue connective tissue stain; × 270. *B*, there has been invasion of the media by lipophages (below and to the right). Sudden death occurred without coronary occlusion in a man aged 34. Frozen section stained with hematoxylin and sudan IV; × 30.

When one considers the association of atherosclerosis with myxedema, the reports of advanced atherosclerosis in cachexia thyreopriva following total ablation of the gland in the early days of surgery of the thyroid gland, the occurrence of atherosclerosis after thyroidectomy in animals, the power of thyroid feeding to prevent atherosclerosis in cholesterol-fed animals, the efficiency in this respect of the administration of iodine in the presence of the thyroid gland and its inefficiency after thyroidectomy, one is led to believe that the mucoid change in the subendothelial layer of the intima may be the result of some form of insufficiency of the thyroid gland that is not marked enough to give rise to the clinical disease myxedema.

(*c*) *Age.*—That age is not a necessary factor is signified by the presence of atherosclerosis in infancy and childhood. The evidence suggests that age becomes a factor in the evolution of atherosclerosis because of inability of the cholesterol metabolism to cope adequately with this substance as the bodily functions become less efficient. The well recognized diminution in functioning of the thyroid gland with age must be considered in this connection.

(*d*) *Stress.*—The localization of the aortic lesions suggests the possible influence of stresses. The usual earliest localization is along the lines of intercostal orifices. This is in the most firmly anchored portion of the aorta. It is possible that the waves of dilation and contraction of the free wall lead to intimal disturbances as they come up against the relatively fixed orifices of the vessels. This may also account for the tendency of lesions to occur about the orifices of the abdominal branches and those of the transverse arch. The lower portion of the abdominal vessel is again firmly anchored and may show more diffuse lesions than other portions. Lesions of the free anterior wall of the thoracic vessel are usually relatively late phenomena. It is known that hypertension favors the development of atherosclerosis. This is particularly exemplified in the coronary arteries. The coronary vessels in the epicardial fat undergo unusual stresses during systole. They are distended and compelled to bear the high aortic pressure, while their myocardial branches are being compressed by the contracting cardiac muscle. Unlike the circulation in other vessels, the main flow through the coronary vessels is during diastole. The unusual stresses account for the frequency of coronary sclerosis, particularly in persons with hypertension. Moreover, the coronary arteries of infants with congenital heart disease, subjected to unusual stresses by the need for an increased blood supply to the cardiac muscle in order to overcome the handicap of the developmental fault, frequently show atherosclerosis. The occurrence of pulmonary atherosclerosis in persons with mitral stenosis is another example of the effect of stress. The subendothelial localization of free

lipoid suggests that widening of the stomas between endothelial cells under stress may furnish access to the lipoid or to monocytes carrying lipoid without endothelial participation in the process.

(*e*) *Infection and Injury.*—In experimental animals it has been shown that infection and injury favor the deposit of cholesterol. The injuries to which the arteries of experimental animals have been subjected are unlikely to be reproduced naturally in man. That infection may favor the localization of atherosclerotic lesions is probable. However, the absence of evidence of inflammatory cellular exudate in the early lesions of atherosclerosis in the experimental animal and in man suggests that this factor, if active, is of relatively minor importance. This is in agreement with the conclusion of MacCallum:[14] "It appears that there is but little evidence in favor of the idea that infections, whether acute or chronic, play a great part in the pathogenesis of arteriosclerosis."

(*f*) *Toxic Factors.*—Nuzum and his associates[15] have suggested the possibility that diets high in protein favor intimal damage. The reports of research in which high protein diets were used in feeding rabbits do not support this thesis. My own studies on rabbits fed the high protein diet of Clarkson and Newburgh gave negative results in this respect. Warren[16] expressed the belief that the excessive and varied osmotic pressure due to the height and irregularity of the blood sugar level in diabetic subjects might possibly lead to injury of the endothelium. Edema of the aortic intima is not a rare process, though it occurs over larger areas than those associated with atherosclerotic lesions and is usually a terminal lesion. There is evidently a considerable degree of permeability of the intima which the media does not usually show, since the edema ceases abruptly at the medial junction, i. e., at the internal elastic lamina. Edema tends to appear, particularly over the surface of atherosclerotic lesions. Its localization and extensive distribution do not correspond to the localization and area of atherosclerotic processes.

ATHEROSCLEROSIS A DISEASE OF THE INTIMA

Thoma advanced the theory that the lesions of atherosclerosis arise in an effort to buttress weak regions in the vessel due to medial faults or damage. He supported this thesis by demonstrating that casts of

14. MacCallum, W. G., in Cowdry, Edmund V.: Arteriosclerosis: A Review of the Problem, New York, The Macmillan Company, 1933, p. 361.

15. Nuzum, F. R.; Seegal, Beatrice; Garland, Ruth, and Osborne, Margaret: Arch. Int. Med. **37**:733, 1926.

16. Warren, Shields: The Pathology of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1930, p. 139.

aortas are cylindric without any depression from arteriosclerotic lesions and that in advanced processes the media may be thinned beneath the lesion. Such a picture is seen in figure 19 *B*. Examination discloses firm scar tissue making up the mass of the lesion and firmly anchored at either end, while the base in the intimal center is wholly necrotic. It is evident that the pull of the scar tissue has compressed the unanchored central necrotic portion against the media and thus produced the thinning. The layers of the media can be followed beneath the lesion and are apparently intact though compressed. In effect the lesion is the cause of the thinning of the media instead of being its result. Support for the Thoma theory is claimed from the results of injection of substances opaque to the x-rays into atherosclerotic arteries (e. g., coronary arteries) post mortem. The vascular shadows are of perfectly cylindric casts, the atherosclerotic nodules being forced back into the wall of the vessel. That these effects are only of postmortem interest is indicated by the results of injection of opaque substances into arteries of living subjects. The tonus of the living vessel is a factor of importance. The arteriogram of an arteriosclerotic vessel of a living subject is characteristic and diagnostic because of the narrowing and irregularity of the vascular shadow.

The sharply localized minute primary initial lesions of atherosclerosis could not in reason arise in relation to and for the purpose of buttressing regions of medial weakness. The examination of early lesions of atherosclerosis, whether in the aorta or in other vessels, indicates that the process is local and primary in the intima.

The surface layers of the media may show early necrosis in the ascending arch (figs. 16 *B* and 17 *A*), invasion of the media by lipoid cells may occur (fig. 20 *B*), to be followed by necrosis (fig. 15 *A*), and scarring may result (fig. 7). Massive scarring of the inner layers many appear in old aortic lesions in relation to atherocheumas. Medial lesions, apart from early necrosis, which is practically limited to lesions in the aortic arch, are secondary to the intimal processes.

The elastica may exhibit marked changes in connection with atherosclerosis, but these changes are secondary to the process in the intima. There may arise fragmentation, flattening and an increase in the elastica.

SUMMARY

All the lesions of aortic atherosclerosis, save the earliest mucoid change, are due to the presence of cholesterol. They are primarily intimal and depend for their nutrition on imbibition through the endothelium. Variation in the character of these lesions is determined by the age of the subject and of the lesions.

In youth cholesterol is introduced into the subendothelial tissue of the intima by globular lipophages or is engulfed by globular lipophages in this situation. Young fibroblastic tissue is produced in the subendothelial tissue, and the young fibroblasts engulf and metabolize the lipoid, leading to its disappearance from the lesions. Repair with minimal scarring follows, since the young fibroblastic tissue does not form collagen.

In middle age cholesterol metabolism within lipoid cells is slowed, the connective tissue forms collagen and scar tissue is produced. There is interference with imbibition of nutriment through the scar tissue, and the deep layers undergo necrosis, with the formation of secondary atheromatous "abscesses" (atherocheumas). Scars are the typical lesions in this period.

In old age cholesterol metabolism ceases, globular lipophages accumulate in masses, with inadequate nutrition and support, and a primary atheromatous "abscess" (atherocheuma) is the typical lesion.

The lesions of the ascending arch are exceptions to these rules, the metabolism of cholesterol being successfully carried on, as in youth, up to advanced ages. The connective tissue which is formed is reticular, as in youth, and minimal scarring is usual.

Calcification arises in connection with necrobiosis or after necrosis has developed. It is a terminal monumental deposit marking the sites formerly occupied by living tissue.

784 Massachusetts Avenue.

ATHEROSCLEROSIS

ETIOLOGY

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CRITICISMS OF EXPERIMENTAL EVIDENCE POINTING TO CHOLESTEROL [1]

Criticisms of the experimental procedure by which atherosclerosis is produced in the rabbit and disagreements with the inferences connecting the results of such experiments with human atherosclerosis have been widely published. In general they can be classified under two heads, as follows:

1. Criticisms have been made of the use of the rabbit as an experimental animal:

(*a*) Because the rabbit never suffers naturally from atherosclerosis. This form of reasoning would exclude Banting's experiments on dogs which gave humanity insulin. It would invalidate most of the animal experimental work dealing with the infectious diseases.

(*b*) Because the diet used is perverted, since rabbits do not naturally ingest cholesterol. While rabbits do not ingest cholesterol they must possess a cholesterol metabolism to care for the needs of their cells, since every animal cell contains cholesterol. From this standpoint cholesterol is not a foreign substance to rabbit tissues. Methods of introducing infectious agents into animals in experimental work in general have been perversions of natural methods, and the agents used have often been foreign to the bodies of the animals used. Perverted diets have been responsible for most of the discoveries with reference to the vitamins. The pathologic effect of the ingestion of vitamin D was demonstrated by marked overdosage, along lines similar to those of experimentation with cholesterol.

(*c*) Because attempts to produce atherosclerosis in the carnivora by feeding cholesterol fail. These efforts fail because the carnivora are able to destroy cholesterol introduced in excess, as Schoenheimer and Breusch [2] demonstrated in the mouse. A natural diet of animal foods, rich in cholesterol, has resulted in the development of an efficient cholesterol metabolism capable of preventing the accumulation of the substance in the tissues and its deposit in arteries.

From the Medical Examiner Service, Suffolk County, Mass.

1. Duff, G. L.: Arch. Path. **20**:81 and 259, 1935.
2. Schoenheimer, R., and Breusch, F.: J. Biol. Chem. **103**:439, 1933.

(*d*) Because the rabbit is far removed from man in the animal scale. This criticism has been met by experimentation on man, as described in a subsequent paragraph.

2. Criticisms have been made that the atherosclerosis of rabbits does not always reproduce the human lesions, as follows:

(*a*) That the deposit of cholesterol is widespread in the experimental rabbit, i. e., in the pulmonary arteries as well as in the systemic arteries, the veins, the reticulo-endothelial system, the cornea and the liver, instead of being limited to the arteries as in the human disease. In answer to this, it should be kept in mind that the rabbit does not naturally ingest cholesterol. As a result its cholesterol metabolism is weak. It is therefore possible to overwhelm this weak metabolism, to induce widespread distribution of the substance and to produce in a few months arterial lesions that it requires many years to produce in man. The faults of this method, i. e., excessive doses of cholesterol in the diet and rapid production, result in lesions which are characterized by excess. This tendency toward excess is encountered in experimental work with many other pathogenic agents.

(*b*) That the arteries of the brain are never affected in the experimental rabbit. The rabbit differs from man anatomically, functionally and posturally. Medlar [3] has found that the localization of experimental tuberculous lesions in the rabbit is influenced by the posture of the animal. The relatively long distance between the brain and the aortic arch, in which the earliest lesions appear in the rabbit, may be a factor, since in the rapidly produced experimental process extension for long distances along branches of the aorta is unusual, though I have seen extension along the carotid artery into the uppermost portion of the long vessel in the neck. The relative importance of cerebral functioning in man (*cogito ergo sum*) and the rabbit, as influencing the requirements for blood and notably for oxygen, may play a part in determining the localization of lesions. It should be remembered that human cerebral sclerosis is a late manifestation, almost without exception.

(*c*) That in the human ascending aortic arch advanced lesions are uncommon while in the experimental rabbit the ascending arch is the site of early and ultimately confluent processes. That this difference in the rabbit's arch is in part due to posture, as commented on in the foregoing paragraph, is probably true. The main distinction is, however, that the lesions of the human ascending arch, while common and multiple, do not usually spread from the initial small processes. The power to metabolize cholesterol in this region at all ages leads to control of the imbibed lipoid, and the absence of collagen formation results in minimal scarring and deformity. When the mechanism which gov-

3. Medlar, E. M.: Personal communication, Am. Rev. Tuberc., to be published.

erns this control is broken down, as by syphilitic aortitis, secondary massive atherosclerotic lesions are usually found. In the rabbit the constant feeding of large doses of cholesterol under experimental conditions apparently overwhelms the protective mechanism in the ascending arch, and the processes extend and become fused into the massive lesions found.

(*d*) That the early lesions in the rabbit may be associated with necrosis of the surface layer of the media, while this does not occur in human lesions. On the contrary, the intimal lesions of the human aortic arch not rarely tend to be linked with necrosis of the surface layer of the media. The appearance of the early human lesions in the arch suggests that this medial necrosis arises at about the same time as the overlying intimal process.

Overaccentuation of the differences and disregard of the similarities between the lesions of the natural and those of the experimental diseases would tend to discredit much of the experimental work on infectious diseases. The too exacting requirement of absolute identity of natural and experimental lesions would be difficult or impossible to satisfy in diphtheria, bacillary dysentery, cholera, meningococcic meningitis, typhoid and syphilis, for example, among others. That the lesions of experimental atherosclerosis in the rabbit correspond closely to human atherosclerotic lesions there can be no reasonable doubt.[4]

EVIDENCE THAT CHOLESTEROL IS THE ETIOLOGIC AGENT

The evidence that atherosclerosis is caused by disturbances in the cholesterol metabolism is as follows:

1. (*a*) Cholesterol is constantly present in the lesions of the disease.

(*b*) It can be isolated from the lesions.

(*c*) When fed in purity to rabbits, it will give rise to the lesions of the disease.

(*d*) It is constantly present in the experimental lesions.

(*e*) No other known agency will produce even suggestive results. Were this a question of a disease of bacterial causation cholesterol would fulfil all requirements as the specific etiologic agent.

2. It has been shown in a preceding paper [5] that the variation in the character of the aortic lesions in atherosclerosis is dependent on the relative ability of phagocytic cells to metabolize cholesterol. In other words, human atherosclerotic lesions apart from the initial mucoid change are produced because of the presence of cholesterol, and the

4. Leary, T.: Arch. Path. **17**:453, 1934. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, p. 271.

5. Leary, T.: Arch. Path., to be published.

varied appearances these lesions present are the results of differences in the reaction to cholesterol of the tissues in which that substance lies, differences which depend largely on the age of the subject or of the lesions.

3. In connection with no other disease has there been such widespread human experimentation in pathogenesis as was carried out by the feeding of diabetic diets high in fat, rich in cholesterol, in the decade 1920-1930. The results were as definite as those obtained in the experimental rabbit. The relative dose of cholesterol was smaller than the standard experimental dose in rabbits, and the results were more slowly produced. The human lesions, while not so spectacular as the more rapidly produced lesions of the rabbits, were spectacular enough,[6] particularly in children.[7] Moreover, the reverse procedure, i. e., the cutting down of the fats in the diet, including cholesterol, resulted in a return to the status quo ante experimentum—i. e., absence of xanthomas and of lesions of the arteries of the leg demonstrable by roentgen examination in children, together with a return to the normal incidence of atherosclerosis in adults.

4. Cholesterol is a substance required by every animal cell. It is an important part of the food intake in the carnivora and a less important part of the diet of the omnivora. It is not ingested by the herbivora and must be synthesized. Presumably the most acute needs for the substance arise at times of most rapid cell division. It is probably for this reason that nature provides in sperm and ova unusual amounts of the substance. Egg yolk, rich in cholesterol, is intended for the embryo. Milk, the fats of which carry considerable cholesterol, is intended for the infant. Milk and its products, eggs and animal fats are the main human sources of cholesterol. Man is the only animal ingesting eggs and milk throughout a lifetime. Man is also the only animal that dies in early life from coronary sclerosis and develops atherosclerosis almost universally with age.

For all of the foregoing reasons it is a justifiable conclusion that atherosclerosis is a disease due to disturbances of the cholesterol metabolism.[8] Stresses appear to be responsible for the localization of the lesions. Aberrations in thyroid function appear to be factors in preparing the intimal ground substance for the deposit of cholesterol.

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6. Warren, Shields: The Pathology of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1930, p. 139.

7. White, Priscilla: Diabetes in Childhood and Adolescence, Philadelphia, Lea & Febiger, 1932, p. 178.

8. The term cholesterol metabolism is used in the broadest sense to include derangements in the tissue exchange of the substance, which may be local or general.

ULTRAVIOLET SPECTROPHOTOMETRIC STUDIES OF HUMAN BLOOD PLASMA

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AND
A. T. SUDMAN, M.D.
OMAHA

The intense absorption of energy by blood proteins in the ultraviolet region and the specificity of absorption spectra have suggested to many the possibility of the application of these phenomena to the recognition of disease. When, in 1927, we began our studies of blood plasma, several such reports had been published. However, on examination of these reports, few if any numerical or graphic data were found. Although the theory of absorption spectra implies unique structural specificity, it was soon recognized that in actual measurement spectrophotometry involves errors of from 1 to 5 per cent. For this reason a considerable portion of our interest was in a study of the available methods.

OPTICAL METHODS

The spectrograph employed was the Hilger E-2 type, and the plates used were chiefly the Wratten & Wainright panchromatic emulsion on a special thin glass. The photometer was the Lewis multiple rotating vane type, using identical sector systems. The room was partially insulated, so that the range of temperature was from 25 to 28 C. The apparatus was mounted on a heavy wooden table, although subsequent experience showed that an all metal frame would have been preferable. Many sources of illumination were tried, including the Pfund iron arc, the carbon arc, the under-water spark of Howe,[1] with some of the modifications suggested by McNicholas,[2] the underwater spark of Henri,[3] Jones electrodes and incandescent lamp sources, such as filaments at overvoltage, pointolites, and the hydrogen tube. Most of the observations on plasma were made with nickel-steel electrodes, and for the power circuit was used a 1 kilowatt, 20,000 volt Acme transformer with the necessary capacity and inductance to give a clear

Aided by a grant from the Committee on Scientific Research of the American Medical Association.

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1. Howe, H. E.: Physical Rev. **8**:674, 1916.
2. McNicholas, H. J.: Bur. Standards J. Research **1**:939, 1928.
3. Henri, V., and de László, H.: Proc. Roy. Soc. London **105A**:662, 1924.

low-pitched tone. The use of synchronous and nonsynchronous gaps, both as sources and as series gaps, as well as a large quenched gap in series did not improve the steadiness of the spark. Cooling the nickel-steel electrodes with large water-jackets helped greatly in maintaining steadiness. The nickel-steel electrodes were made by milling a knife edge 0.031 inch (7.9 mm.) in thickness on the ends of rods 0.25 inch (6.4 mm.) in diameter. The spark-gap mounting was constructed from the base of a Bausch & Lomb slit lamp. The changes permitted (*a*) the recording of the *x*, *y* and *z* positions of the gap, (*b*) the vertical adjustment of the electrodes by a micrometer and (*c*) the insertion of new electrodes in a predetermined position.[4]

Observations on the spark agreed well with the statement by Harrison that the tone is the best guide to proper action, except for the actual measurement of intensity. However, direct observation and photography with suitable filters showed that a condensed spark is a curved band running between the edges of the gap and that such a spark passes around the edges of the gap, stopping at various places for irregular periods. Occasionally these variations in position occurred without noticeable change in tone. Attempts to decrease the area of wandering by thinning the knife edges resulted in excessive heating and arcing. The use of air blasts, single, double, parallel and at right angles to the spark, was of negligible value. It was thought that a fine stream of water through one electrode, impinging on the other, would furnish a straight path for the spark. This was found to be true, but the spattering was undesirable.

Filament sources are unsuitable, since radiation at temperatures of much below 5,000 K. (Kelvin) does not give adequate emission in the ultraviolet region. The radiation of tungsten near its melting point produces intense emission in the visible region and almost none below 2,700 angstroms.[5]

During the past three years we have used essentially a duplicate of the Lawrence and Edlefsen hydrogen tube.[6] We have dispensed with the large water container and have water-cooled merely the constricted tube, using a fan and metal radiators for cooling the electrodes. The power was furnished by a 5 kilovolt ampere transformer with a primary of 110 and a secondary of 3,300 volts. With an input of from 10 to 20 amperes ample illumination was obtained. Except for breakage, this source of illumination has been satisfactory.

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4. Sudman, A. T.: Ultra-Violet Spectrophotometry of Biological Fluids, Thesis, University of Nebraska, 1931.

5. Forsythe, W. E., and Christison, Frances: J. Optic. Soc. America **20**:396, 1930.

6. Lawrence, E. O., and Edlefsen, N. E.: Rev. Scient. Instruments **1**:45, 1930.

The alinement of the apparatus was accomplished by several methods, which in our hands proved superior to the method suggested by Hilger. The suggestion by McNicholas that a beam of light be passed in a reverse direction through the optical train was useful.[2] However, final adjustment usually required a series of test plates. This was easily done with the rigid mounting for the nickel-steel gaps. The short focus of the Lewis photometer makes its alinement more critical than that of instruments of longer focus, although Baly and his associates [7] have shown that the Lewis photometer is as accurate as instruments of longer focus. This is in accord with our experience.

The Lewis photometer is self-calibrating, but reliance was placed mostly on a test absorption plate. We prepared a set of optical flat plates of Corning G-984B glass, calibrated by the Bureau of Standards No. 55,065. Chart 1 records all the data obtained for an eight-month period, no observations being omitted. The symmetrical irregularities which are most conspicuous at the peaks are characteristic of shifts in the position of the spark. Many of these were noted at the time the photographs were taken. In the work on plasma most of these irregularities were avoided as we became familiar with the operation of the gap, and they did not occur in the studies in which the hydrogen tube was used. It should be pointed out that the calibration curve for the test plates prepared by the Bureau of Standards is a graphically smoothed curve with a reported estimated error of transmission of from ± 0.01 to ± 0.03.

PREPARATION OF MATERIAL

Specimens of normal blood were obtained from healthy laboratory workers. Pathologic specimens were taken from patients with the following conditions, who were selected for the clinical purity of the disease: carcinoma of the stomach, malignant hypertension, exophthalmic goiter, cholangeitis with jaundice, typhoid, toxic adenoma of the thyroid, lobar pneumonia, miliary tuberculosis, acute tuberculosis, pernicious anemia and lymphatic leukemia. The clinical report of these cases has been made by Sudman.[4]

All the specimens of blood were obtained in the morning twelve or more hours after the last feeding. Heparin was used in the amount of 2 mg. per ten cubic centimeters of blood. Heparin showed slight absorption in the region of the spectrum studied, but this was negligible in the amounts used. The heparin commercially available gives with Folin's reagent a slight reaction to phenol, which is said to disappear with further purification. We were unable to obtain the highly purified product. Specimens showing hemolysis, lipemia or turbidity were discarded. Plasma was used in preference to serum because serum is a syneretic fluid, although Suhrmann and Kollath [8] and others have made observations with serum which correspond well with those shown by the plasma curves in our

7. Baly, E. C. C.; Morton, R. A., and Riding, R. X.: Proc. Roy. Soc., London, s.B1 **113**:709, 1927.

8. Suhrmann, R., and Kollath, W.: Strahlentherapie **27**:572, 1928.

series. Samples were photographed as soon as possible, usually within two hours. The cells used by Lewis were found to be the most satisfactory.[9] These consisted of two plane quartz plates 1 inch (2.54 cm.) square, in parallel, separated by a thickness of tin-foil of U shape and held together with spring clamps. Separators of glass of the size desired were too fragile, while brass separators resulted in occasional precipitation with marked change in absorption. The usual depth of the cell was 0.11 mm. The thickness of each cell was measured with a precision micrometer for every filling, the thickness of the fluid being the difference between the two quartz plates alone and the plates and the separator. Any change in the

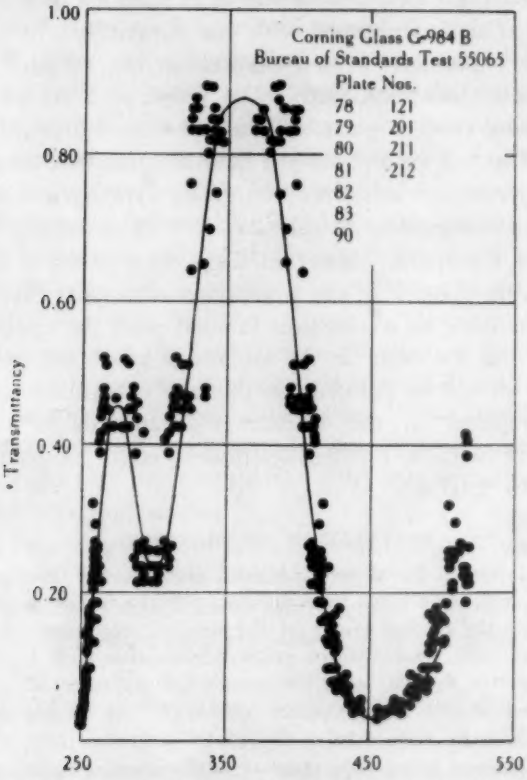

Chart. 1.—All the observations on a test plate for a period of eight months, a condensed spark between nickel-steel electrodes being used as the source. The symmetrical irregularities are due to erratic shifts in the position of the spark. In most cases these were recognizable by changes in tone at the time the photograph was made. The solid line is the graphically averaged curve of the Bureau of Standards. The ordinates represent wavelengths expressed in millimicrons.

depth of the cell on repeated assembly was accounted for before the cell was used. The separators of tin-foil were flattened by pressing them between two flat scraped metal surfaces. The quartz end-plates were checked with an optical flat plate.

9. Hilger, Adam: Personal communication to the authors.

The time of exposure varied from five seconds to two minutes. Test plates were made with each specimen, and the plasma was checked for possible deterioration due to age or to excess exposure to light.

The plates were read at first with a microscope comparator, green light being used. Later a stereopticon projector was used. This was more satisfactory, since there was less eyestrain and two observers could work together.

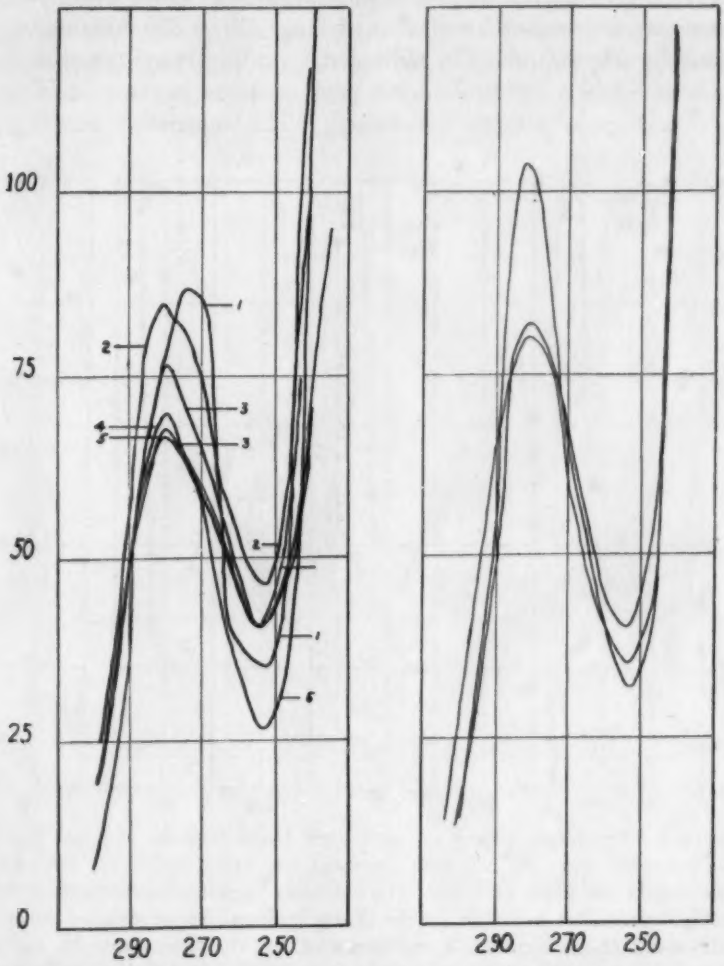

Chart 2.—Comparison of absorption curves for normal blood serums. Curve 1 was obtained by Henri;[8] curve 2, by Lewis;[10] curve 3, by Stenström and Reinhard;[11] curve 4, by Suhrmann and Kollath,[8] and curve 5, by Schweizer.[15] The curves are drawn from published graphs and not from numerical data. The curves on the right are for normal plasma.

RESULTS

The results are shown in the accompanying charts. There was a slight variation in the wavelength of the maximum in both the normal

and the pathologic plasmas, the range being from 280 to 282 millimicrons for the normal plasmas, as compared with that of from 279 to 284 millimicrons for the pathologic plasmas. The peak of 284 millimicrons was found in a case of malignant hypertension, the rest of the peaks being 282.5 millimicrons or below. Similarly, the range of the wavelengths of the minimum absorption was from 249.5 to 252.5 millimicrons, as compared with that of from 249 to 256 millimicrons for the pathologic group, the 256 millimicron reading being taken in a case of typhoid, while a 255 millimicron peak occurred in the case of malignant hypertension already mentioned. The remaining readings for

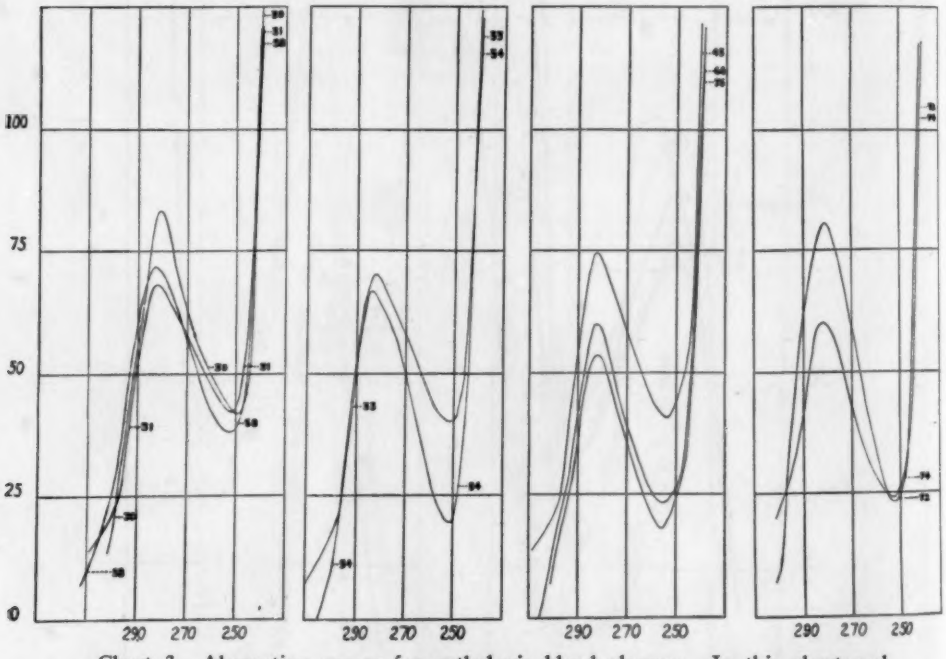

Chart 3.—Absorption curves for pathologic blood plasmas. In this chart and in the following chart the ordinates represent extinction coefficients for undiluted plasma, with a cell depth of 1 cm. The abscissas represent wavelengths expressed in millimicrons. The numerals on the charts indicate curves obtained in cases of the following disease: curve 30, carcinoma of the stomach; curve 31, malignant hypertension; curve 58, lobar pneumonia; curve 33, exophthalmic goiter; curve 54, toxic adenoma of the thyroid; curve 45, lymphatic leukemia; curve 66, typhoid; curve 76, pernicious anemia; curve 72, miliary tuberculosis, and curve 74, acute pulmonary tuberculosis.

pathologic plasmas were 253 millimicrons or below. Inspection of the curves reveals no significant variations in contour, and, as has already been pointed out, the method does not permit a reliable determination of slight changes in the slope of the curves. There was no characteristic

variation in the heights of the maxima or minima in the diseases studied. Occasional changes in slope found on single plates were due to instrumental errors and largely disappeared on the check plates.

The marked constancy of the end-absorption below 245 millimicrons, as well as the relative constancy of that above the maximum, is to be noted. The zone of greatest variation was between the maximum and the minimum, and at times seemingly true variations in slope occurred in this region, but they appear too slight to be reliable criteria for selection.

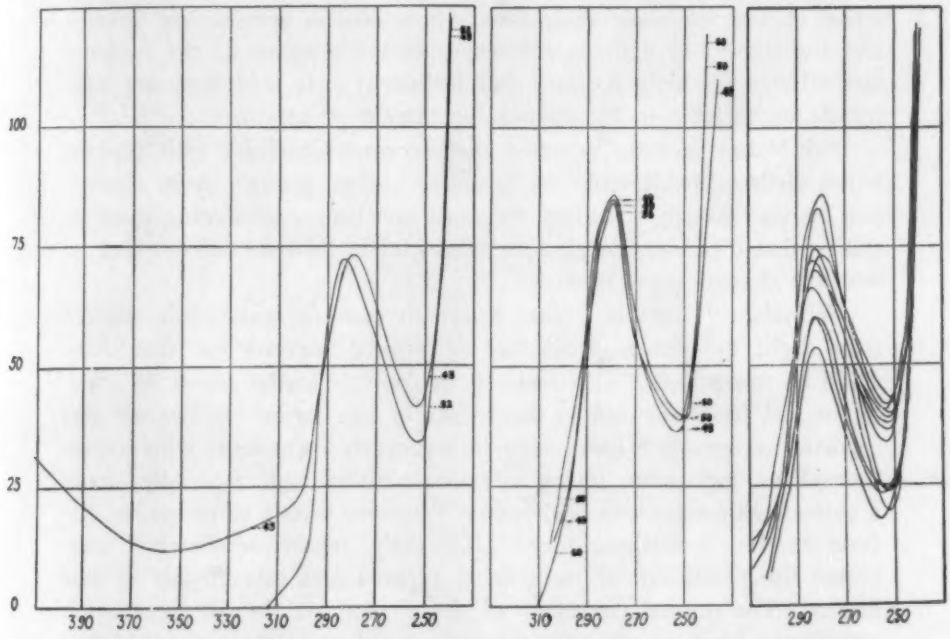

Chart 4.—Absorption curves for blood plasmas. Curve 43 is the absorption curve in a case of cholangeitis with icterus; curve 52, the curve in the same case two weeks later, and curves 49, 50 and 60, curves in a case of typhoid. The superimposed curves at the right are all the curves shown in charts 3 and 4.

COMMENT

The constancy of the absorption curves for the normal plasmas and for plasmas from persons with the diseases studied and the marked similarity to the absorption curves for serum proteins leave little doubt that the predominant curve for blood plasma and blood serum is due to these substances. Svedberg and Sjögren [10] found maxima and

10. Svedberg, T., and Sjögren, Bertil: J. Am. Chem. Soc. **52**:2855, 1930.

minima at 280 and 252 millimicrons for serum albumin and at 284 and 254 millimicrons for serum globulin, as compared with corresponding values of 281 and 252 millimicrons for the normal and pathologic plasmas, respectively, in our series. That tyrosine and tryptophan contribute largely to the curve has been emphasized repeatedly, but Stenström and Reinhard [11] were unable to make a mixture of amino-acids which gave an absorption curve having more than a gross resemblance to that for plasma, except at a p_H of 12.7. Uric acid did not affect the absorption in the material we examined. Although Krupski and Almasy [12] concluded that uric acid is an important factor in blood serum curves, the weak absorption of uric acid in protein-free filtrates and the slight but definite differences in the position of the maxima and minima certainly indicate that ordinarily uric acid does not contribute appreciably to the curves for plasma or serum.

Paic [13] has recently reported that serum of patients with syphilis is not distinguishable from the serum of normal persons by its absorption curve. Kehar [14] reached the same conclusion concerning malaria, stating that the absorption is somewhat greater than normal but that no selective changes were observed.

Schweizer [15] concluded that serum in cases of carcinoma showed only slight differences from that of normal persons but that these could be recognized. This result is in contrast to the report of Stenström and Reinhard and to the results in our series. Schweizer also reported recognizable differences in serums from patients with hyperthyroidism, mongolian idiocy and osteomalacia and, especially, from a patient with a sulfuric acid burn. We were unable to recognize differences for hyperthyroidism. The early report of Lewis [16] suggested the possibility of recognizing typhoid and tuberculosis by this means. The normal variations of the method are, in our experience, sufficiently large to preclude the interpretation of these small differences as specific for disease. In a study of absorption spectra for rabbits for periods of six months, the range of variation in the intensity of the bands was considerable.[17] Variation in the absorption for groups of normal persons is not related to hemoglobin content, erythrocyte or leukocyte counts, sex, age or blood type.

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11. Stenström, K. W., and Reinhard, M.: J. Biol. Chem. **66**:819, 1925.
12. Krupski, A., and Almasy, F.: Naturwissenschaften **19**:461, 1931.
13. Paic, M.: Compt. rend. Acad. d. sc. **198**:286, 1934.
14. Kehar, N. D.: Rec. Malaria Survey India **3**:171, 1932.
15. Schweizer, P.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **38**:343, 1925.
16. Lewis, S. J.: J. Soc. Arts **69**:799, 1921.
17. Dunn, F. Lowell, and Sudman, A. T.: J. Bact., to be published.

CONCLUSIONS

1. There are no significant changes in the absorption spectra of untreated blood plasma in the region of from 220 to 310 millimicrons in cases of typhoid, tuberculosis, pernicious anemia, exophthalmic goiter, toxic adenoma, cholangeitis, malignant hypertension, lobar pneumonia, lymphatic leukemia, carcinoma of the stomach, syphilis or malaria.

2. Further refinements in apparatus may record differences in the region of from 250 to 290 millimicrons.

CARDIOVASCULAR AND ARTHRITIC LESIONS

IN GUINEA-PIGS WITH CHRONIC SCURVY AND HEMOLYTIC STREPTOCOCCIC INFECTIONS

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In 1933 Rinehart and Mettier [1] described cardiac and arthritic lesions in scorbutic guinea-pigs with coexisting streptococcic infections and directed attention to certain points of resemblance between these lesions and those of rheumatic fever. The purpose of the experiments described here was to study further in guinea-pigs the pathologic effects, particularly in the cardiovascular system, of chronic scurvy and of chronic infection with hemolytic streptococci when present separately and when acting synergically. Coincidentally it was the purpose to determine the relative degree of scurvy and the type of infection requisite to the production of characteristic cardiovascular lesions.

Cardiac lesions have been observed in most histopathologic studies of scurvy in guinea-pigs. Holst and Frölich [2] frequently observed fatty degeneration of the myocardium; and Höjer,[3] reviewing the reports of others and summarizing the results of his own investigations on scorbutic animals both with and without infection, stated that the myocardium frequently shows "atrophy combined with necrosis which has a tendency to calicification." He also noted a "weakness" of the perivascular elastic substance in the heart. Many subsequent investigations have yielded similar results. In a recent study in which attention was directed toward lesions other than those of the osseous system, Bessey, Menten and King [4] noted extensive foci of fatty degeneration in the myocardium.

The combined effect of scurvy and infection also has been extensively investigated (recently reviewed by Robertson [5]), but histologic examinations have been made infrequently Findlay [6] produced chronic scurvy by giving 2.5 cc. of orange juice weekly to guinea-pigs deprived of other sources of vitamin C and infected them with pneumococci or

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1. Rinehart, J. F., and Mettier, S. R.: Am. J. Path. **9**:932, 1933.
2. Holst, A., and Frölich, T.: Ztschr. f. Hyg. u. Infektionskr. **72**:1, 1912.
3. Höjer, J. A.: Acta pædiat. (supp.) **3**:8, 1924.
4. Bessey, O. A.; Menten, M. J., and King, C. G.: Proc. Soc. Exper. Biol. & Med. **31**:455, 1934.
5. Robertson, E. C.: Medicine **13**:123, 1934.
6. Findlay, G. M.: J. Path. & Bact. **26**:1, 1923.

staphylococci. The animals survived up to fifteen days, but fatty degeneration of the myocardium was the only change observed in the heart. Rinehart and Mettier [7] have studied the pathologic changes in the joints and hearts of scorbutic guinea-pigs with especial reference to the problem of rheumatic fever. They examined the organs of animals with acute, subacute and chronic scurvy when uncomplicated and also after infection (chiefly with hemolytic streptococci) had been induced. In uncomplicated scurvy minimal effects were observed in the joints and in the valves of the heart, but in animals with coexistent infection there were distinct changes "fundamentally similar" to those of rheumatic fever. The lesions described included accumulations of "hyalin fibrinous material" within joint capsules, areas of "eosinophilic hyalin" in subsynovial, capsular and periarticular tissues, as well as hyperplasia and proliferation of synovial linings. Also, proliferative lesions associated with areas of fibrinoid degeneration were present in the valves of the heart and in the myocardium.

Stimson, Hedley and Rose [8] have reported recently the production of similar myocardial proliferations (no fibrinoid change was described) in acutely scorbutic guinea-pigs following intracardiac injection of the toxin produced by a strain of hemolytic streptococcus derived from a patient with scarlet fever.

In the experiments described here an attempt was made to discover the effects of various definite grades of chronic scurvy, both uncomplicated and combined with spontaneous or induced chronic infection with hemolytic streptococci.

METHODS

Animals.—Male and female guinea-pigs [9] of mixed breeds were used; most of them weighed between 300 and 350 Gm.

Diet.—Water and the basal diet (containing supplementary food factors except vitamin C) described by Rinehart and Mettier [7a] were supplied ad libitum. After a period of two weeks the animals were maintained in various grades of chronic scurvy by supplementing the ration with constant weekly allowances of 2, 6 or 9 cc. of orange juice, respectively, given by pipet in divided doses thrice weekly. Controls received the basal diet plus 5 cc. of orange juice daily or rations of hay, oats and cabbage.

Infection.—All artificially infected animals were inoculated with one strain (J20) of hemolytic streptococcus recovered from a guinea-pig with spontaneous lymphadenopathy (belonging to group C of hemolytic streptococci according to

7. Rinehart, J. F., and Mettier, S. R.: (*a*) Am. J. Path. **9**:952, 1933; (*b*) **10**:61, 1934. (*c*) Rinehart, J. F.; Connor, C. L., and Mettier, S. R.: J. Exper. Med. **59**:97, 1934.

8. Stimson, A. M.; Hedley, O. F., and Rose, E.: Pub. Health Rep. **49**:361, 1934.

9. Mouriquand, G., and Michel, P.: Compt. rend. Soc. de biol. **90**:231, 1924.

the serologic classification of Lancefield [10]). The cocci were grown for twenty hours in "streptococcus broth," [11] and 0.2 cc. of culture was injected subcutaneously into the right groin. Spontaneously infected guinea-pigs were taken from a stock in which purulent lymphadenopathy was rampant. Blood agar plates inoculated with pus from either the spontaneous or the induced lesions showed pure growth of hemolytic streptococci. The colonies were about 2 mm. in diameter and mucoid, with fairly wide zones of hemolysis.

Pathologic Technic.—The animals which did not succumb to infection were killed by a blow below the occiput. A microscopic examination of the heart was made in every instance, and in certain groups the aorta, all the costochondral junctions of one side and the left (uninfected side) knee joint also were included. The lungs were examined microscopically when the diagnosis of pneumonia was in question. The tissues were fixed in Zenker's solution, sectioned in paraffin and stained with eosin and methylene blue and the Van Gieson elastica stain. Bones were decalcified with 10 per cent nitric acid. Before being embedded each aorta was rolled in such a manner that the entire longitudinal extent was represented in each section. Several sections were made from each of the two blocks of the heart—one included the aortic valve and the left ventricle and the other the mitral valve (occasionally the tricuspid) and portions of both ventricles.

DEGREES OF SEVERITY OF CHRONIC SCURVY INDUCED

The several grades of chronic scurvy induced corresponded to the weekly supplements of 3, 6 or 9 cc. of orange juice. An attempt was made to estimate by different methods the relative degree of scurvy at each of these levels of dosage.

(*a*) *Rate of Growth of Teeth.*—Dalldorf and Zall [12] have found that the retardation in the rate of growth of teeth in scorbutic guinea-pigs constitutes a reliable index of the severity of the disease. By their technic, the left lower central incisor was clipped every five days, and the rate of growth of the stump was measured. The results are tabulated briefly in table 1. The rates of growth corresponding to different levels of the dosage of orange juice agree closely with those determined by Dalldorf and Zall. Judged by these criteria, the degree of scurvy in animals receiving 9 cc. of orange juice weekly was indeed "slight." On the other hand, those receiving 3 cc., while designated as having "moderate scurvy," showed a retardation of the growth of the teeth approximating that in animals with complete scurvy. The "mild scurvy" incident to weekly doses of 6 cc. of orange juice represented an intermediate grade.

(*b*) *Changes at the Costochondral Junctions.*—The pathologic changes at the costal junctions also were studied in several groups in order to determine the relative degrees of scurvy present. In evaluating

10. Lancefield, R. C.: J. Exper. Med. **57**:571, 1933.

11. Swift, Homer F., and Hodge, B. E.: Proc. Soc. Exper. Biol. & Med. **30**: 1022, 1933.

12. Dalldorf, G., and Zall, C.: J. Exper. Med. **52**:57, 1930.

the results, the criteria of Holst and Frölich,[2] Hess,[13] Wolbach and Howe[14] and Delf and Tozer[15] were followed. The results in all the animals studied from this point of view are summarized in table 2. Pathologic conditions at the costochondral junctions varied in severity corresponding to the degree of the restriction of orange juice. No differences, however, could be discerned between infected and noninfected animals receiving equivalent quantities of orange juice. The costochondral junctions of eight nonscorbutic guinea-pigs were examined microscopically, and of these only three showed slight alterations, such as occur in scurvy. Each of the five animals receiving 9 cc. of orange juice weekly presented slightly more definite changes. There were some shortening and irregularity of the columns of cartilage cells

TABLE 1.—*Rates of Growth of Teeth with Different Dosages of Orange Juice in Chronic Scurvy*

Number of Guinea-Pigs	Period of Observation, Days	Weekly Supplement of Orange Juice, Cc.*	Average Daily Growth, Mm.	Designation
12	16th–20th	0	0.31	Acute scurvy†
3	26th–30th	3	0.34	Moderate scurvy†
3	41st–45th	3	0.38	Moderate scurvy†
9	17th–21st	6	0.43	Mild scurvy†
3	26th–30th	9	0.60	Slight scurvy
3	41st–45th	9	0.65	Slight scurvy
4	26th–30th	30	0.76	No scurvy
4	41st–45th	30	0.78	No scurvy
3	.....	0‡	0.85	No scurvy

* The basal diet was given without supplement in each instance from the first to the fourteenth day.

† Terms employed by Dalldorf and Zall (after Sherman) designating a similar rate of growth of the teeth.

‡ Diet of cabbage, oats and hay.

in the epiphyseal cartilage, with a reduction in the size of the individual cells and occasional irregularity of the corticalis. An increased proliferation of the osteogenic zone of the periosteum was inconstantly noted. The slenderness, irregularity and decrease in number of the osseous trabeculae were definite in a few instances. In seven animals which received 6 cc. of orange juice weekly the changes just described were more constant, and in addition a decrease in the number of lymphoid cells was evident in the marrow at the end of the diaphysis, while in two instances the marrow in this region over very small areas was replaced by a reticulated matrix. Each of the four animals receiv-

13. Hess, A. F.: Scurvy Past and Present, Philadelphia, J. B. Lippincott Company, 1920.

14. Wolbach, S. B., and Howe, P. R.: Arch. Path. & Lab. Med. **1**:1, 1926.

15. Delf, E. M., and Tozer, F. M.: Biochem. J. **12**:416, 1918.

ing only 3 cc. of orange juice weekly showed definite evidence of chronic scurvy. The corticalis was thin, irregular and occasionally fractured, while the bony trabeculae were in some instances reduced to fragments, and over considerable areas the marrow at the end of the epiphysis was replaced by a cellular matrix. Figure 10 represents an example of the changes noted.

TABLE 2.—*Changes Noted at the Costochondral Junctions in Scorbutic and Non-scorbutic Guinea-Pigs Both With and Without Infection*

Guinea-Pig Number	Weekly Supplement of Orange Juice, Cc.	Presence of Infection	Epiphyseal Cartilage			Corticalis			Osseous Trabeculae		Marrow at End of Epiphysis				Intercostal Myositis
			Cell Columns Short	Cell Columns Irregular	Cells Small	Thin	Irregular	Proliferation in Osteogenic Zone of Periosteum	Decreased Number	Slender and Irregular	Decreased Number of Lymphoid Cells	Decreased Number of Blood Vessels	Blood Extravasation	Matrix Present	
621	30 (or diet of cabbage, oats and hay)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
586			0	±	+	0	±	-	±	0	0	0	+	0	0
625			0	±	0	0	±	0	±	0	0	0	0	0	0
550			0	0	0	0	0	0	0	0	0	0	0	0	0
551			0	0	0	0	0	0	0	0	0	0	0	0	0
553			+	0	+	0	0	0	0	±	0	0	0	0	0
552		+	+	0	+	0	±	+	+	0	0	0	0	0	0
684			0	0	0	0	0	0	0	0	0	0	0	0	0
638	0	0	+	+	+	0	0	+	+	±	0	0	0	0	++
612			+	±	+	0	0	0	±	+	0	0	0	0	++
607			+	+	+	0	+	+	+	+	0	0	+	0	++
609		+	+	+	+	0	+	+	+	±	0	0	0	0	++
610			0	0	+	0	0	0	0	0	0	0	0	0	0
540	6	0	+	+	+	+	±	+	+	+	±	0	+	0	+
554			++	+	+	+	+	±	++	±	+	0	++	+	+++
556			+	+	+	±	+	++	±	+	±	0	0	0	++
545		0	0	0	+	0	±	++	±	+	0	0	0	0	+++
540		+	+	+	+	±	+	+	++	+	0	0	+	0	++
639			++	+	+	+	++	+	+	+	+	+	0	+	++++
606			±	0	+	0	±	+	+	±	±	±	0	0	++
770	3	0	++	++	+	++	+++	++	+++	+	++	+	+	++	++++
616			±	+	+	+	+	0	++	++	+	+	++	+	+++
768		+	+	+	+	++	++	+	+++	++	±	0	0	0	+++
772			++	++	+	++	+	+	+	++	++	0	+	++	+++

(*c*) *Degree of Intercostal Myositis.*—Dalldorf [16] observed that the nature of the lesions of the skeletal muscles (especially the intercostal muscles) in scorbutic guinea-pigs varies with the character of the accompanying changes in the bones, and this offers an additional means of estimating the severity of the disease. Table 2 indicates that in this series the intercostal myositis increased in severity with the progressive reduction in the quantity of orange juice supplied, and its occurrence sharply differentiated those animals with the mildest degree of chronic

16. Dalldorf, G.: J. Exper. Med. **50**:293, 1929.

scurvy from the controls. There were opacity, fracture and marked eosinophilia of occasional muscle fibers and fiber groups adjacent to areas of loose, collagen-poor replacement connective tissue. Large histiocytes were sometimes present. An area of intercostal myositis is represented in figure 10.

The differences found in the rate of growth of teeth, in the osseous structures of the costochondral junctions and in the severity of intercostal myositis permit an estimate of the degree of chronic scurvy occurring with the different amounts of orange juice administered. Thus, 9 cc. given weekly permitted a just perceptible degree of chronic scurvy; 3 cc., chronic changes of considerable severity, and 6 cc. weekly, an intermediate grade. Following in part the nomenclature employed by Dalldorf [12] and Sherman,[17] these grades are designated here as follows: The basal diet plus 9 cc. of orange juice daily produced "slight" chronic scurvy; the basal diet plus 6 cc. of orange juice daily, "mild" chronic scurvy, and the basal diet plus 3 cc. of orange juice daily, "moderate" chronic scurvy.

Clinical signs of scurvy were not evident in animals with slight chronic scurvy. The animals with mild chronic scurvy showed a distinct disinclination to move, while the animals with moderate involvement were often inactive and frequently assumed the characteristic "face ache" position. None of the uninfected scorbutic animals died.

CHARACTER OF THE INFECTION IN NONSCORBUTIC GUINEA-PIGS

Local subcutaneous abscesses with enlargement of the adjacent inguinal lymph nodes developed in guinea-pigs inoculated subcutaneously in the groin. These lesions persisted for many weeks, and in about half the animals transient discharging sinuses appeared. Of the twenty-six artificially inoculated nonscorbutic guinea-pigs from an uninfected stock (table 4), seven died; in five of these and in four others that were killed purulent inflammation of internal organs was evident macroscopically at autopsy.

In four of twenty-four control guinea-pigs (tables 3 and 4, acute spontaneous infection) from an uninfected stock, acute spontaneous infection with hemolytic streptococci developed while the animals were under observation, and one of them died. There were small subcutaneous abscesses in two animals, while the other two showed pulmonary abscesses at autopsy.

Four young animals were selected from a stock in which spontaneous lymphadenopathy due to infection with hemolytic streptococci

17. Sherman, H. C.; La Mer, V. K., and Campbell, H. L.: J. Am. Chem. Soc. **44**:165, 1922.

was prevalent. Cervical abscesses developed in all of them and regressed in two instances; but in none of the animals was there any involvement of the internal organs, and none of them died (table 4, chronic spontaneous infection).

CHARACTER OF THE INFECTION IN SCORBUTIC GUINEA-PIGS

In scorbutic guinea-pigs infected experimentally larger, more edematous local and subcutaneous lesions developed than in nonscorbutic animals (table 5), and the mortality rate was much higher, although the internal organs were not more frequently involved in purulent inflammation. Of twenty-three scorbutic guinea-pigs artificially inoculated, fifteen died, and in seven there was purulent disease of the internal organs. There was a definite correlation between the degree of scurvy

TABLE 3.—*Cardiac Data for Twenty Uninfected Nonscorbutic Guinea-Pigs*

		Endocarditis			Myocarditis		Pericarditis					Valvulitis		
	Purulent Carditis	Mononucleare	Mononucleare and Eosinophils	Proliferation	Mononucleare	Muscle Degeneration	Mononucleare	Mononucleare and Eosinophils	Nodular Proliferation	Fibrinoid Change	Perivascular Lesions	Edema	Degeneration	Proliferation
Number of animals showing respective changes.....	0	9	2	0	5	1	5	1	0	0	6	0	0	0

present and the mortality rate from induced infection: over half of those with slight scurvy survived, while all the infected animals with moderate scurvy succumbed.

Acute spontaneous infection with hemolytic streptococci developed in four of the fifteen scorbutic guinea-pigs from "uninfected" stock (table 5) while they were under observation. None of them showed subcutaneous lesions, but they all died as a result of a purulent involvement of visceral organs.

Moderate chronic scurvy was induced in four guinea-pigs selected from a stock in which spontaneous lymphadenopathy due to infection with hemolytic streptococci was prevalent (table 5, chronic spontaneous infection). Large cervical abscesses developed, but the animals lived for over a month. In three of them there was purulent inflammation of internal organs.

In both scorbutic and nonscorbutic guinea-pigs, therefore, as a consequence of experimental infection of the type employed, there was a

TABLE 4.—*Data for Nonscorbutic Guinea-Pigs with Induced and Spontaneous Hemolytic Streptococcic Infection*

Guinea-Pig Number	Duration of Scurvy, Days	Duration of Infection, Days	Number of Animals Died or Killed	Extent of Local Inflammation	General Data Induced Infection	Purulent Carditis	Endocarditis			Myocarditis		Pericarditis			Fibrinoid Change	Perivascular Lesions	Valvulitis		
							Mononuclears	Mononuclears and Eosinophils	Proliferation	Mononuclears	Muscle Degeneration	Mononuclears	Mononuclears and Eosinophils	Nodular Proliferation			Edema	Degeneration	Proliferation
(26 guinea-pigs)	0	4 to 160	7 D 19 K	0 in 5, + to ++++ in 21	Number of animals*	6 + 20 0	9	1	0	3	1	4	2	0	0	6	4	0	0
					Acute Spontaneous Infection														
552	0	60—	K	+	Small inguinal abscess with sinus	0	0	++	0	0	0	0	+	0	0	+	0	0	0
585	0	?	K	0	Lung abscesses,† pneumonia	0	0	+	0	0	0	++	+	0	0	0	0	0	0
621	0	21±	K	0	Inguinal abscess‡	0	0	+	0	0	0	0	+	0	0	0	0	0	0
684	0	?	D	0	Lung abscesses,† pneumonia	0	0	0	0	0	0	0	0	0	0	0	+	0	0
					Chronic Spontaneous Infection														
763	0	60+	K	+	Inguinal abscess‡	0	0	0	0	0	0	0	0	0	0	0	0	0	0
764	0	60+	K	++++	Large cervical abscess†	0	0	+	0	0	0	0	0	0	0	0	0	0	0
765	0	60+	K	++++	Large cervical abscess†	0	0	+	0	0	0	+	0	0	0	+	0	0	0
766	0	60+	K	+	Small cervical abscess with sinus‡	0	0	+	0	0	0	0	0	0	0	0	0	0	0

* In instances of purulent carditis no attempt is made to indicate the character and relative severity of any associated cardiac lesions.

† Hemolytic streptococci recovered in pure culture from the lesion.

‡ A large inguinal abscess was present earlier during the period of observation.

TABLE 5.—*Data for Infected and Uninfected Guinea-Pigs with Various Degrees of Scurvy* *

Guinea-Pig Number	Duration of Scurvy, Days	Duration of Infection, Days	Died or Killed	Extent of Local Inflammation	General Data	Purulent Carditis	Endocarditis			Myocarditis		Pericarditis			Fibrinoid Change	Perivascular Lesions	Valvulitis		
							Mononuclears	Mononuclears and Eosinophils	Proliferation	Mononuclears	Muscle Degeneration	Mononuclears	Mononuclears and Eosinophils	Nodular Proliferation			Edema	Degeneration	Proliferation
					Slight Scurvy (9 Cc. of Orange Juice per Week)														
615	53	0	K	0	.....	0	+	0	0	0	0	0	0	0	0	0	0	+	+
638	53	0	K	0	.....	0	+	0	0	+	0	0	0	0	0	0	0	0	+
612	57	0	K	0	.....	0	+	0	0	0	0	0	0	0	0	0	0	0	0
461	43	8	D	++++	.....	3+	P	P	P	P	P	P	P	P	P	P	P	P	P
492	46	11	D	+	.....	3+	P	P	P	P	P	P	P	P	P	P	P	P	P
450	42	14	D	++	.....	3+	P	P	P	P	P	P	P	P	P	P	P	P	P
607	53	28	K	0	Abscess †	0	2+	0	0	0	+	+	0	0	0	+	0	0	+
609	53	28	K	+	Abscesses: ‡ chest wall, lung, mediastinum	0	0	0	0	0	0	0	+	0	0	0	0	0	0
608	60	35	K	++	Lung abscesses,‡ pneumonia	0	+	0	0	+	0	0	0	0	0	0	0	+	0
610	60	35	K	++++	.....	0	+	0	0	0	0	+	0	0	0	+	0	0	+
					Mild Scurvy (6 Cc. of Orange Juice per Week)														
549	84	0	K	0	Scurvy §	0	+	0	0	0	0	+	0	0	0	0	0	0	2+
554	87	0	K	0	Scurvy §	0	+	0	0	0	0	0	0	0	0	0	0	0	+
556	86	0	K	0	Scurvy §	0	+	0	0	0	0	0	+	0	0	0	0	0	+
557	84	0	K	0	Scurvy §	0	+	0	0	0	0	0	+	0	0	0	0	0	+
463	43	8	D	++++	.....	3+	P	P	P	P	P	P	P	P	P	P	P	P	P
546	80	13	D	++++	Scurvy §	3+	P	P	P	P	P	P	P	P	P	P	P	P	P
547	81	14	D	++++	Scurvy §	3+	P	P	P	P	P	P	P	P	P	P	P	P	P

611	26	?	D	0	Acute spontaneous infection, lung abscesses ‡	0	0	0	0	+	0	0	0	0	2+	0	0	3+	0
555	86	?	K	0	Scurvy,§ acute spontaneous infection, hepatic abscesses ‡	0	0	3+	3+	0	0	0	2+	0	0	0	0	+	4+
488	33	5	D	+++		0	2+	0	2+	3+	3+	0	0	0	0	0	0	2+	2+
489	39	11	D	++++		0	0	0	0	0	0	0	0	0	0	0	+	0	0
539	80	34	D	++++	Abscess ‖	0	0	0	0	+	2+	+	0	0	0	+	0	0	2+
545	84	38	K	+++	Abscess ‖	0	+	0	0	0	0	+	0	0	0	0	0	0	2+
540	86	40	K	++++	Abscess ‖	0	2+	0	0	0	0	0	4+	3+	3+	3+	0	3+	4+
639	86	40	K	0	Abscess ‖	0	3+	0	2+	3+	2+	0	0	0	0	0	0	0	0
606	87	41	K	0	Abscess ‖	0	+	0	0	0	0	0	0	0	0	0	0	0	0
					Moderate Scurvy (3 Cc. of Orange Juice per Week)														
770	40	0	K	0		0	+	0	0	0	0	0	0	0	0	0	0	0	0
616	48	0	K	0	Marked dehydration; moribund; cultures negative	0	0	3+	0	0	0	3+	0	0	0	2+	0	0	+
617	53	0	K	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0
618	58	0	K	0		0	0	0	0	0	0	+	0	0	0	+	0	0	0
541	63	17	D	++++	Splenic abscesses,‡ peritonitis,‡ pleurisy,‡ pneumonia,‡ pericarditis ‡	3+	P	P	P	P	P	P	P	P	P	P	P	P	P
548	16	?	D	0	Acute spontaneous infection, retroperitoneal abscess ‡	0	0	0	0	2+	2+	3+	0	0	2+	3+	0	3+	+
614	17	?	D	0	Acute spontaneous infection, lung abscesses,‡ pneumonia ‡	0	0	0	0	0	0	2+	0	2+	+	0	0	0	+
456	32	4	D	++		0	0	0	0	3+	0	0	0	0	0	0	0	0	0
455	40	5	D	++++		0	0	0	0	0	0	0	0	0	0	0	0	0	+
538	52	6	D	+++		0	0	0	0	0	0	0	0	0	0	0	0	0	0
491	36	8	D	+++		0	0	0	0	0	0	0	0	0	0	0	0	0	+
544	57	11	D	++		0	0	0	0	2+	2+	+	0	2+	2+	+	0	+	+
767	30	30+	D	++++	Chronic spontaneous infection, peritonitis‡	0	+	0	0	0	0	+	0	0	0	0	0	0	+
773	35	35+	D	++++	Chronic spontaneous infection, pleurisy,‡ pneumonia	0	0	0	0	+	0	0	0	0	0	0	0	0	+
768	37	37+	D	+	Chronic spontaneous infection, lung abscesses ‡	0	0	0	0	0	0	+	0	0	0	0	0	0	+
772	37	37+	D	++++	Chronic spontaneous infection	0	0	0	0	0	0	+	0	0	0	0	0	2+	2+

* In the instances of purulent carditis no attempt is made to indicate the character and relative severity of any associated cardiac lesion. "P" indicates present.

† A large inguinal abscess was present earlier during the period of observation.

‡ Hemolytic streptococcus recovered in pure culture from the lesion.

‖ A large inguinal abscess was present earlier during a period of moderate scurvy.

§ Moderate scurvy was present for a period earlier when there was no clinical sign of infection.

tendency toward the development of localized chronic inflammation. Acute spontaneous infection developing in uninfected stock animals resulted more frequently in purulent visceral disease, especially in scorbutic animals. Chronic spontaneous infection remained localized and tended to heal spontaneously in nonscorbutic guinea-pigs. In animals with moderate scurvy, however, such lesions did not regress, and there was extension of purulent inflammation to the viscera, with eventual death.

These types of purulent lymphadenitis due to infection with hemolytic streptococci have been described by Theobald Smith.[18] His experiments demonstrated that incident to the development of herd immunity a relatively benign localized type of the disease results. The virulent strains causing the epidemics which he studied, however, engendered rapidly fatal infections even when spread on the uninjured skin of guinea-pigs from uninfected stocks. The factor of herd immunity was probably operative in determining the type of disease which has been termed chronic spontaneous infection in the experiments described here.

CARDITIS

(*a*) *Controls.*—Table 3 concerns twenty nonscorbutic uninfected control guinea-pigs. Three received the basal diet plus an adequate supplement of orange juice, and the remainder received a diet of cabbage, oats and hay. The criteria for the absence of infection were as follows: continuous gain in weight, absence of clinical signs on semiweekly examination and negative macroscopic postmortem observations, supplemented by a microscopic study of sections of the lungs in many instances. Half the animals in this group presented no discernible cardiac lesions, while in the other half the changes were minimal. Most commonly seen were small compact localized accumulations of mononuclear cells (sometimes associated with a few eosinophils) in the mural endocardium and occasionally extending beneath it, but without evidence of damage to adjacent myocardial fibers. Such foci were multiple and were observed in the walls of all the chambers, although only a few were seen in one heart; a predilection for the papillary muscles of the left ventricle was evident. Similar collections were occasionally present in the myocardium, where they were limited to a few mononuclear cells, occasionally perivascular in location. Pericardial lesions were invariably perivascular and usually consisted of a few sparsely scattered lymphocytes and mononuclear cells; in one instance eosinophils also were present. The only other cardiac lesions presented in the control group were occasional small recent myocardial hemorrhages.

18. Smith, T.: Internat. Clin. **3**:276, 1931.

(*b*) *Infection Without Scurvy.*—Three main types of chronic infection with hemolytic streptococci were studied: (1) induced, (2) acute spontaneous and (3) chronic spontaneous infection. In table 4 the nonscorbutic infected animals are grouped according to this classification. In the hearts of thirty-four infected animals lesions were observed similar to those in the control animals. Although they were slightly more extensive here, a discernible difference in the character of the lesions was evident only in the instances of spontaneous infection. In those cases eosinophils were invariably present in the small cellular accumulations occasionally noted in the mural endocardium. Acute focal myocarditis or myocardial abscesses were present in seven instances, five of them fatal cases. The only other unusual feature was slight valvular edema.

(*c*) *Scurvy Without Infection.*—The criteria for the absence of infection were the same as those for the nonscorbutic uninfected group, with the exception that here failure to gain weight was not considered of significance in this connection. In the hearts of eleven guinea-pigs with uncomplicated scurvy (table 5) lesions similar to those in the control group were observed. In addition, diffuse valvular degeneration with slight proliferation was not uncommon (figs. 3 and 4). More intense localized valvular proliferative reactions were occasionally seen, but an extensive change of this character was encountered only once (guinea-pig 616 of the group with moderate scurvy). In this instance there were also fairly extensive myocarditis and pericarditis with perivascular inflammation. The animal was moribund when killed, showing marked dehydration and cachexia, but no definite evidence of the presence of infection was observed.

(*d*) *Scurvy with Infection.*—Slight Scurvy: In this group of seven guinea-pigs (table 5) there were three instances of acute focal myocarditis resembling in all respects those in the nonscorbutic infected animals. For the rest, lesions were of a character and extent duplicated in animals with uncomplicated scurvy of comparable severity. These animals were all experimentally infected.

Mild and Moderate Scurvy: In the group with mild scurvy there were ten guinea-pigs experimentally infected and two which had acute spontaneous infection. In the group with moderate scurvy there were six animals experimentally infected, two with acute spontaneous infection and four with chronic spontaneous infection. The hearts of the four guinea-pigs with chronic spontaneous infection and moderate scurvy showed only changes of the type seen in uncomplicated scurvy. In four instances in which infection was induced focal purulent myocarditis of the usual type was present.

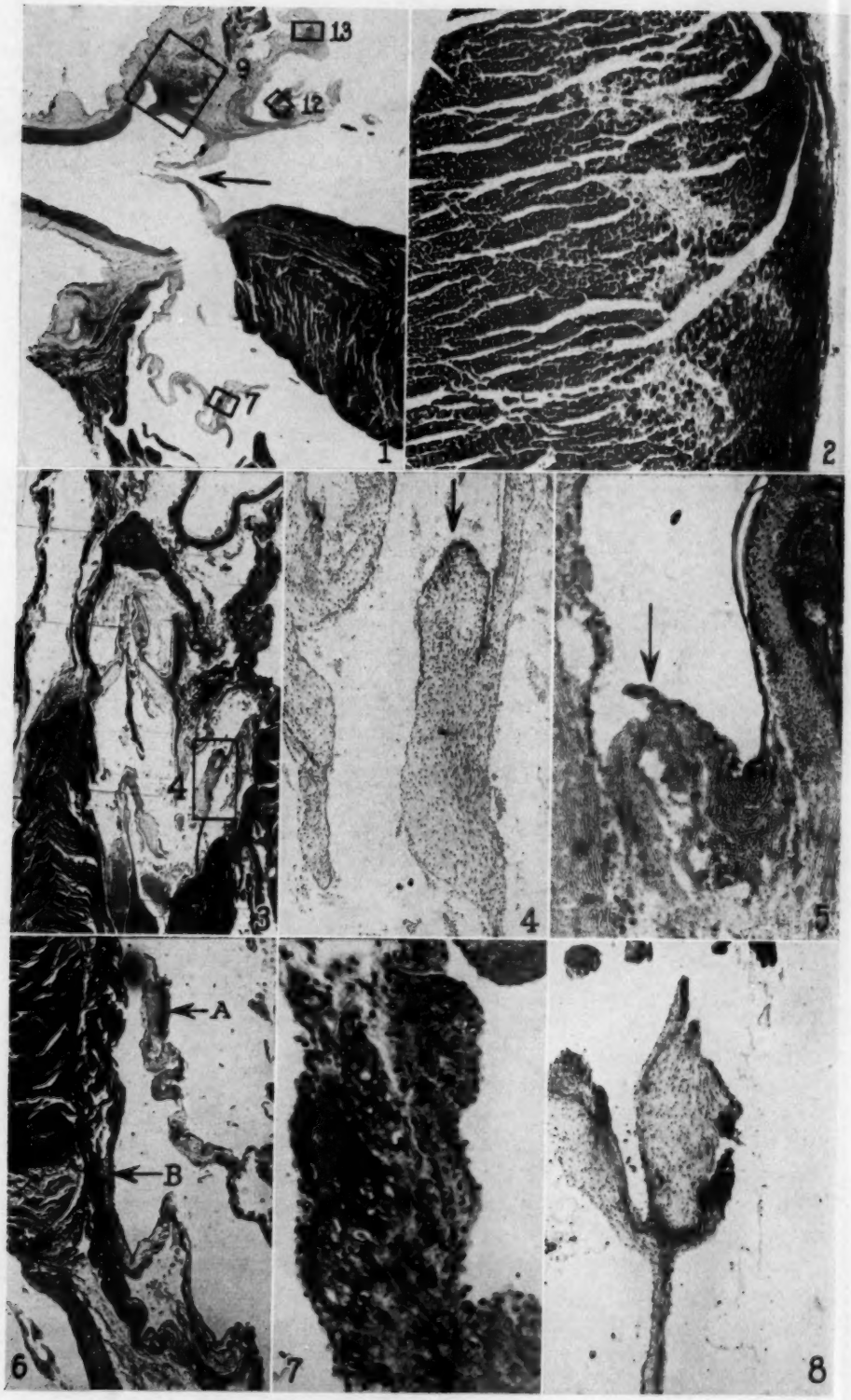


PLATE I

Explanation of Plate I

Fig. 1.—Heart; guinea-pig 540. Mild scurvy had been present for eighty-six days, and induced infection, for forty days. The distribution of the lesions is shown, and areas *7*, *9*, *12* and *13* are reproduced with higher magnification in the figures of corresponding numbers. The arrow indicates a proliferative lesion of the aortic valve. Magnification, × 8.

Fig. 2.—Heart; guinea-pig 639. Mild scurvy had been present for eighty-six days, and induced infection for forty days. There is destruction of the myocardial fibers with connective tissue replacement. Magnification, × 42.

Fig. 3.—Heart; guinea-pig 615. Slight scurvy had been present for fifty-three days (uninfected). There is slight proliferative thickening of the valves. The area at *4* is shown with higher magnification in figure 4. Magnification, × 8.

Fig. 4.—Heart; guinea-pig 615. Enlargement of area *4* in figure 3. There are thickening of the valve, incident to proliferation of the stroma and slight endothelial proliferation at the point indicated. Magnification, × 42.

Fig. 5.—Knee joint; guinea-pig 768. Moderate scurvy had been present for thirty-seven days, and chronic spontaneous infection, for a longer period of time. A fibrinoid mass in a joint recess is shown in which a few lymphocytes and large mononuclear cells are enmeshed. Magnification, × 45.

Fig. 6.—Heart; guinea-pig 555. Mild scurvy had been present for eighty-six days; acute spontaneous infection had been of unknown duration. At *A* is seen a leaflet of the mitral valve with a small area of fibrinoid degeneration and surrounding proliferative reaction. At *B* is seen the proliferative thickening of the mural endocardium. Magnification, × 16.

Fig. 7.—Tricuspid valve; guinea-pig 540. Mild scurvy had been present for eighty-six days, and induced infection, for forty days. Enlargement of area *7* in figure 1, showing fibrinoid degeneration.

Fig. 8.—Mitral valve; guinea-pig 611. Mild scurvy had been present for twenty-six days, and there was acute spontaneous infection of unknown duration. Proliferative thickening with a degenerative lesion at the line of closure and destruction of endothelium is seen. Magnification, × 45.

TABLE 6.—*Pathologic Changes in the Knee Joints of Scorbutic Guinea-Pigs Both With and Without Infection*

Degree of Scurvy	Guinea-Pig Number	Duration of Scurvy, Days	Duration of Infection, Days	Died or Killed	General Data	Non-purulent Carditis	Peri-articular Myositis	Synovial Proliferation	Material (Fluid) in Capsule	Hyalinoid Change	Osseous Scurvy
Mild	549	84	0	K	No infection	±	+	+	0	0	0
	554	87	0	K		±	+++	+	0	0	0
	556	86	0	K		±	++++	0	0	0	0
	557	84	0	K		±	+++	0	0	0	0
	555	86	?	K	Acute spontaneous infection.....	+++	++	+	0	0	0
	545	84	38	K	Induced infection	+	+++	+	0	0	0
	540	86	40	K		+++	++	0	0	0	0
	639	86	40	K		++	+++	0	0	0	0
	606	87	41	K		±	++	±	0	0	0
Moderate	616	48	0	K	No infection	++	++	+	+	+	+
	618	58	0	K		±	+++	+	0	0	+
	541	63	17	D	Induced infection (purulent carditis)..	Purulent carditis	++++	±	0	+	++
	614	17	?	D	Acute spontaneous infection.....	++	+	+	+	0	++
	538	52	6	D	Induced infection.....	0	+++	?	0	0	+
	544	57	11	D		+++	++	+	0	+	++
	773	35	35+	D	Chronic spontaneous infections	±	++	+	+	0	+
	768	37	37+	D		±	+++	+	+	+	+++
	772	37	37+	D		±	+	+	+	0	++

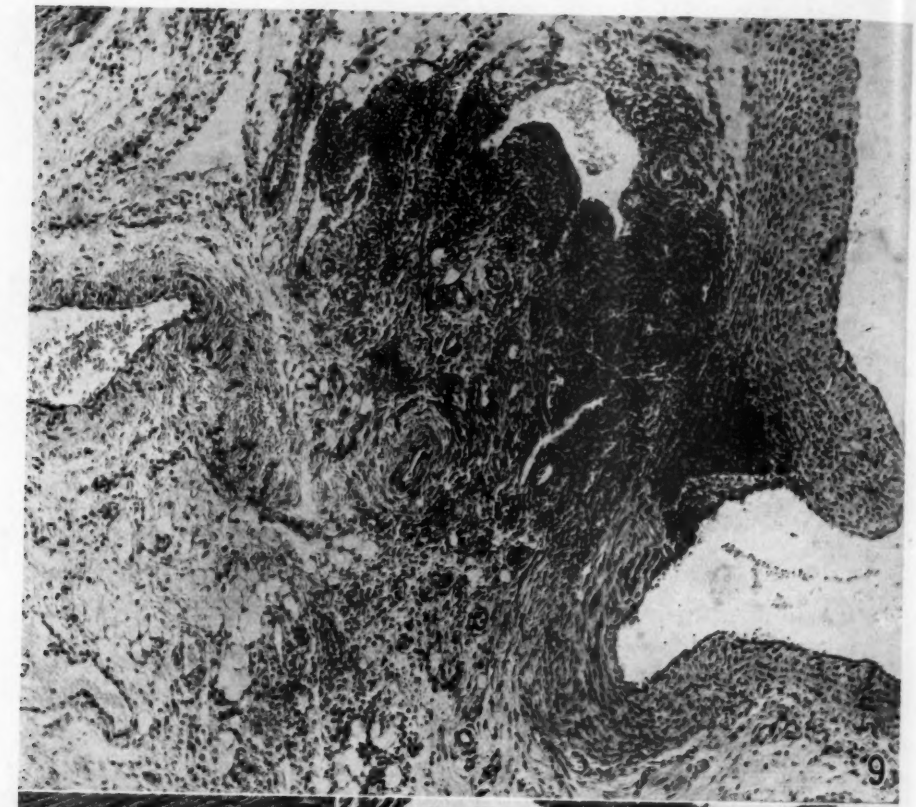
In the groups of animals with mild and moderate scurvy, however, other experimentally infected animals and those with acute spontaneous streptococcic disease characteristic cardiac lesions developed. Areas of fibrinoid degeneration were present in the valves (figs. 6, 7, 8, 11 and 13) and in the pericardium, occasionally in perivascular areas. In those lesions the connective tissue was homogeneous, waxy in appearance and deeply eosinophilic. The character of the cellular reaction varied. Occasionally only a few shrunken, deeply staining nuclei of connective tissue cells remained; at other times large, pale mononuclear cells were noted occupying clear areas in the eosinophilic matrix (fig. 7), and in some instances there were fairly dense accumulations of large mononuclear cells about the borders (fig. 13). Areas of degeneration staining with methylene blue were also common, particularly in the valves, and were usually associated with rather compact collections of mononuclear cells. The two manifestations of localized degeneration were occasionally observed to be adjacent. There were occasionally areas in which the valvular endocardium was destroyed, but no concomitant thrombus formation was evident (fig. 8).

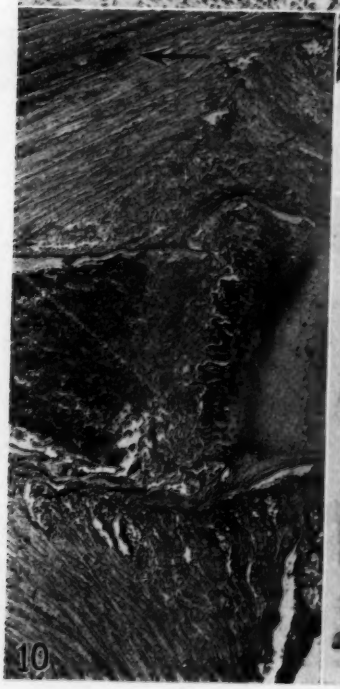
Valvular proliferations of extreme degree were observed (figs. 6, 12 and 13). The entire structure of the valve was sometimes obscured by densely packed large mononuclear cells (fig. 12), but usually at least a strand of eosinophilic material was visible in such lesions (figs. 6, 11 and 13). Proliferations of the mural endocardium were occasionally seen (fig. 6).

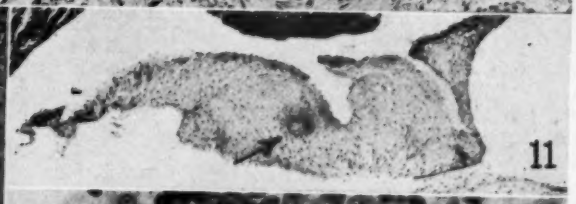
Myocarditis was noted infrequently. Sometimes there were areas of loose connective tissue surrounding deeply eosinophilic necrotic muscle fibers. In other myocardial lesions (fig. 2) the connective tissue was more cellular and the destruction of muscle less extensive. Rarely, small mononuclear cells were diffusely scattered through the myocardium. Nodular proliferations in perivascular regions were occasionally seen, chiefly in the epicardium. They were uniformly composed of mononuclear cells, some of them with little cytoplasm and round or oval, deeply staining nuclei and others with diffuse cytoplasm and large, irregular, deeply staining nuclei and several distinct nucleoli. A few polymorphonuclears were usually present.

AORTITIS

Although no macroscopic lesion was apparent in any aorta, microscopic examination of several specimens from each group frequently demonstrated the presence of slight aortitis. The inflammation usually was not extensive and consisted of accumulations of mononuclear cells and eosinophils, either localized or distributed in a seam at the junction between the media and the adventitia. Occasionally, similar cells were







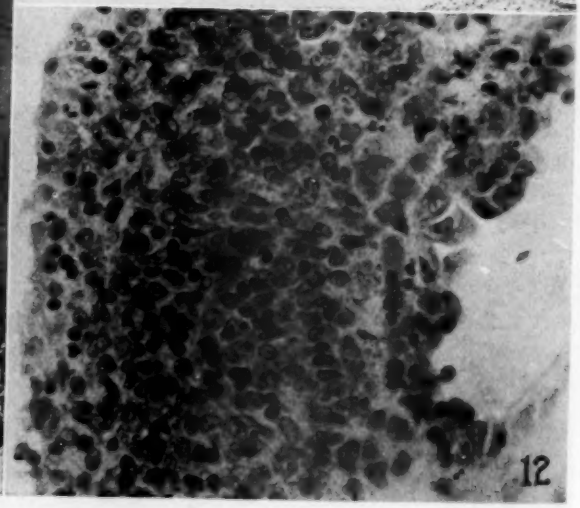


PLATE II

EXPLANATION OF PLATE II

Fig. 9.—Root of the aorta; guinea-pig 540. Mild scurvy had been present for eighty-six days, and induced infection, for forty days. Enlargement of area *9* in figure 1, showing a nodular lesion in the epicardium with extension into the media of the aorta. Magnification, × 45.

Fig. 10.—Costochondral junction; guinea-pig 770. Moderate scurvy had been present for forty days, but no infection. The columns of cartilage cells are short and disorganized; the osseous trabeculae are few and deformed. The arrow indicates an area of intercostal myositis. Magnification, × 18.

Fig. 11.—Mitral valve; guinea-pig 548. Moderate scurvy had been present for sixteen days; there was acute spontaneous infection of unknown duration. An area of localized degeneration with a sparse accumulation of large mononuclear cells and lymphocytes is seen. Magnification, × 18.

Fig. 12.—Mitral valve; guinea-pig 540. Mild scurvy had been present for eighty-six days; induced infection, for forty days. Enlargement of area *12* in figure 1. The entire width of the valve is included, showing the types of large mononuclear cells involved. Magnification, × 360.

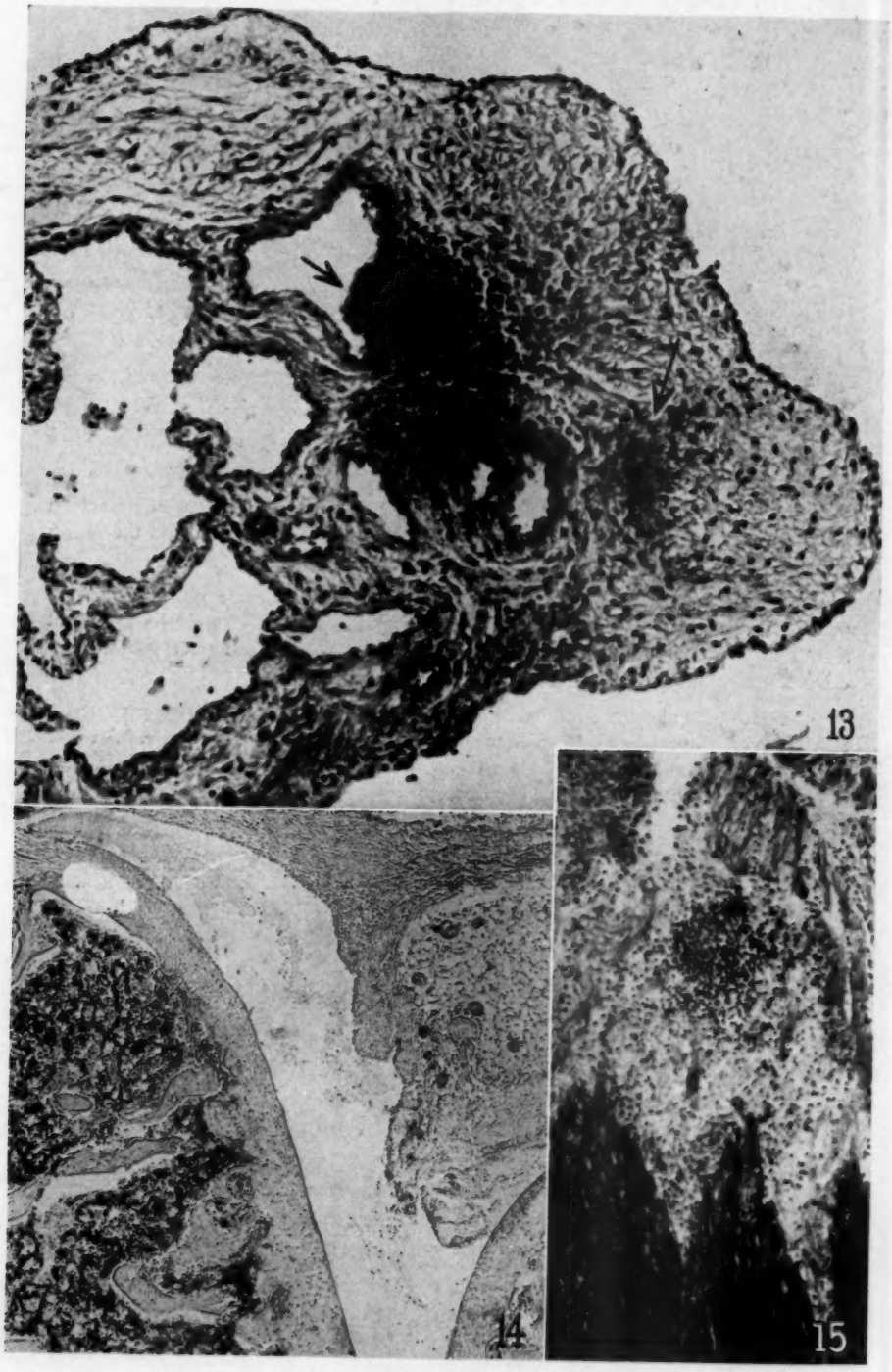


PLATE III

EXPLANATION OF PLATE III

Fig. 13.—Mitral valve; guinea-pig 540. Mild scurvy had been present for eighty-six days; induced infection, for forty days. Enlargement of area *13* in figure 1, showing proliferative thickening of the valve with two areas of fibrinoid degeneration, one attended by an intense cellular reaction. Magnification, × 77.

Fig. 14.—Knee joint; guinea-pig 773. Moderate scurvy had been present for thirty-five days; chronic spontaneous infection, for a longer period of time. The joint space contains amorphous material. Magnification, × 42.

Fig. 15.—Myocardium; guinea-pig 639. Mild scurvy had been present for eighty-six days; induced infection, for forty days. There is degeneration of myocardial fibers about a nodular accumulation of lymphocytes and large mononuclear cells. Magnification, × 77.

diffusely scattered in the adventitia, but in such instances their occurrence could be accounted for by the presence of inflammation in a neighboring organ, e. g., pneumonia. There was no disease of the vasa vasorum or involvement of the external portion of the media. Rarely, in the intima and inner third of the media degenerative changes were evident. This slight degree of aortitis occurred in members of each of the groups, and its character and severity apparently were not influenced by the presence of either scurvy or infection.

ARTHRITIS

Clinically, slight swelling and stiffness were noted in the knees of guinea-pigs with moderate scurvy. There were thickening and induration of periarticular tissue, but the joint capsules were not distended with fluid. The involvement was apparently confined to muscles in the region of the articulation. Only in those instances in which there was direct extension of inflammation from a neighboring abscess was the joint modified by the presence of infection. In artificially infected animals edema and induration extending distally from an abscess in the left groin to the region of the left knee were frequently observed.

The knee joints of eighteen guinea-pigs with either mild or moderate scurvy, some with and some without infection, were examined microscopically. In the case of infected animals the knee from the uninfected (right) side of the body was selected for study. The observations are summarized in table 6. In the neighborhood of the joints there was severe and extensive myositis which corresponded to the type noted in the intercostal muscles. Slight thickening and proliferation of the synovial lining was inconstantly present in the animals with mild scurvy but occurred rather regularly in animals with moderate scurvy. In the latter, small fibrin clots enmeshing a few lymphocytes and endothelial cells were occasionally seen free in the joint spaces or adherent to synovial or cartilaginous surfaces; and in the joint recesses similar masses in which some hyalinoid change had apparently taken place sometimes fused with or replaced the synovia. Such changes are illustrated in figure 5. Osseous lesions characteristic of scurvy and analogous to those described at the costochondral junctions were discernible in animals with moderate scurvy. These changes occurred principally at the ends of the diaphyses and were sometimes associated with subperiosteal hemorrhages. No areas of typical fibrinoid degeneration were apparent, and there was no evidence of periarticular inflammation except in the muscles. The arthritic lesions were not more severe or extensive in the infected than in the uninfected scorbutic guinea-pigs.

COMMENT

The circumstances most conducive to the development of the characteristic cardiac lesions may in part be defined by studying the data presented in table 5. The degree of chronic scurvy present must be at least mild or moderate. There is probably a narrow margin between the severity of scurvy essential to the development of lesions in the presence of chronic infection with hemolytic streptococci and that which does not permit survival of the scorbutic animal under such circumstances. The requisite duration of infection may be only from five to eleven days (guinea-pigs 488 and 544). The severity of focal infection required cannot be closely defined, because the minimal response was a large inguinal abscess and in several such instances characteristic cardiac lesions developed (e. g., guinea-pigs 540 and 544).

It is evident that the nature of the infection is a determining influence. In guinea-pigs *767*, *773*, *768* and *772* (table 5) the most extensive local and visceral purulent lesions were present, and these animals were subjected to the most severe degree of chronic scurvy for over one month. Despite this, the cardiac lesions were of slight degree. These were animals with chronic spontaneous infection. In contrast to this group were guinea-pigs 611, 555, 548 and 614, the scorbutic animals with acute spontaneous infection. In this group of animals, two of which were subjected to only mild scurvy, the most extensive and highly characteristic cardiac lesions developed. The difference in the behavior of these two groups cannot be accounted for by the presence of more extensive purulent visceral involvement in one or entirely by a difference in the duration of the combined action of scurvy and infection. For example, in the animals with late spontaneous infection the scurvy had been present for from thirty to thirty-seven days, while in three of those with early spontaneous disease the duration of a combined effect of scurvy and infection could not have exceeded twenty-six days, although in one the interval may possibly have been eighty-six days. The animals in which infection was induced stand in an intermediate position with reference to the two groups just described.

These observations indicate that spontaneous infection arising during the course of chronic scurvy is slightly more effective than induced infection in giving rise to characteristic cardiac lesions. Of greater significance, however, is the fact that the continuance of even a severe infection during chronic scurvy is relatively ineffective in inducing carditis if it has become well established *before* the induction of scurvy. It therefore appears that an important factor involved in the development of characteristic cardiac lesions is the state of immunologic reactivity of the animal during the period in which scurvy and infection coexist.

A comparison of the characteristic lesions in infected scorbutic animals with those of patients with rheumatic fever is of interest. The outstanding experimental cardiac lesion was a focal interstitial valvulitis with areas of fibrinoid degeneration; there were occasional instances of focal nonpurulent myocarditis. On the other hand, no verrucous endocarditis was seen, and the architecture of the myocardial lesions did not closely resemble that of Aschoff bodies. In each affected heart the lesions were few and were not distributed as are the multiple granulomas of rheumatic fever.

A synergic effect of scurvy and infection in producing arthritis was not observed. The scorbutic arthritic lesions were not granulomatous, and there was no marked exudative reaction, such as occurs in rheumatic fever. The myositis in infected scorbutic animals appeared to duplicate that of uncomplicated scurvy and was not of an infectious type.

Chronic streptococcic infection in guinea-pigs is associated with a state of bacterial hyperergy of the tuberculin type.[19] The resulting altered vulnerability of the tissues to injury incident to the presence of scurvy is possibly responsible for the lesions described. These probably additive effects have not, in my opinion, induced changes characteristic of rheumatic fever.

CONCLUSIONS

Chronic scurvy and chronic infection with hemolytic streptococci acting synergically may induce nonpurulent carditis in guinea-pigs. Valvulitis with fibrinoid degeneration and an intense proliferative reaction constitutes the most prominent lesion. The changes only slightly resemble those seen in cases of rheumatic fever.

For the production of such lesions chronic scurvy at least of mild degree (resulting from a restriction of the vitamin C intake to an equivalent of 6 cc. of orange juice weekly) is requisite.

Both spontaneous infection with hemolytic streptococci in guinea-pigs and that induced in them by inoculation with a strain originally isolated from the lesions of animals with this disease are effective synergically in inducing carditis if their onset does not antedate the development of chronic scurvy, but they are ineffective in this respect if scurvy is induced after a certain degree of antistreptococcic immunity has been established.

Chronic scurvy uncomplicated by infection induces proliferative lesions of minimal extent.

In chronic scurvy of even mild degree there is extensive myositis of the intercostal muscles and of the muscles in the neighborhood of the knee joints. In more severe grades of chronic scurvy slight arthritic

19. Moen, J. K.: Unpublished work.

changes are demonstrable. These manifestations are apparently unaffected by the presence of infection.

Small localized areas of endocarditis and myocarditis were seen in about half of the control guinea-pigs. There were lesions of similar character and distribution in scorbutic and in infected animals, although in the latter they were slightly more extensive and eosinophils were more frequently present.

Slight aortitis was occasionally noted in all groups of guinea-pigs examined. The lesions were apparently not affected by the presence of scurvy, infection or a combination of the two processes.

FACTORS DETERMINING NECROSIS OR SURVIVAL OF LIVER TISSUE AFTER LIGATION OF HEPATIC ARTERY

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BROOKLYN

Ligation of the hepatic artery may result in necrosis of the liver, or the liver may survive wholly or in part. The explanation of these facts has seemed clear, especially after the publications of Haberer;[1] that is, necrosis occurs if anastomoses are absent, and the liver is preserved when and in areas in which adequate anastomoses are present (Zimmerman[2] and Cameron[3]).

According to this theory, arterial blood is indispensable for the preservation of the liver. To this one may object that even normally the portal vein supplies the liver with more than three fourths of the necessary blood (Schwiegk[4]) and that portal venous blood is not free from but only poor in oxygen, its oxygen content being 20 per cent less than that of arterial blood (Wright[5]). The blood of the hepatic artery, reaching the liver lobules, is for the most part not arterial but venous. Thus, only a small number of the capillaries of the hepatic artery are thought to reach the lobules of the liver directly (Olds and Stafford[6] and Kastert[6a]), and the existence of even these is denied by many authors (Kaufmann[7]). Lack of arterial blood with the portal vein patent, bringing blood to the liver as usual, may conceivably result in any kind of degeneration, such as fatty change; such sudden, rapid, acute necrosis, however, has not been explained satisfactorily and demands renewed investigation.

From the Department of Pathology, the Crown Heights Hospital.

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EXPERIMENTAL INVESTIGATION

Rats and rabbits were used in the following experiments. The employment of rats was planned originally only as a makeshift, but it proved necessary to extend the experiments with their use.

Each series of experiments, except the last, was repeated from six to ten times and conducted for periods varying from two days to three or four weeks.

I shall describe the experiments briefly and report the results only so far as necrosis is concerned. Changes in the liver of any other sort, such as disturbances in the metabolism of fat, glycogen, etc., will not be taken into consideration.

Adult rats, weighing from 150 to 200 Gm., were used. Narcosis was effected by intraperitoneal injection of from 0.7 to 0.8 cc. of a 10 per cent solution of sodium amytal in distilled water. The narcotic is effective in from five to ten minutes and can be supplemented with a few whiffs of ether. Death does not occur from narcosis, provided the doses mentioned are not exceeded. Absolutely sterile technic is not necessary in rats. Infections do not occur, except occasional suture suppurations of the skin, which heal quickly and may be disregarded.

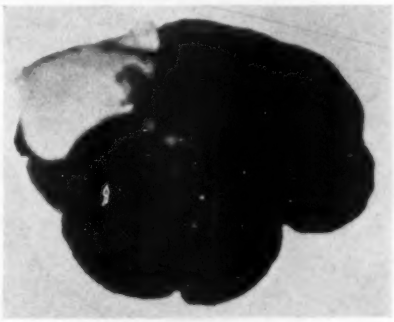

Fig. 1.—Liver of a rat into which india ink was injected two days after ligation of the hepatic artery and a branch of the portal vein to the right lateral lobe. The liver appears black except for the lobe supplied by the ligated vessel. A small gray-black area shows the extension of anastomoses to the neighboring lobe.

Ligation of the Main Branch of the Portal Vein.—All the rats died within from two to six hours or were found dead the next morning. The liver was intact, with no necrosis.

Ligation of the Main Trunk of the Hepatic Artery.—The artery was ligated close to the liver in order to exclude possible anastomoses. All the rats survived and were under observation for four weeks after operation. The liver showed marked fatty changes in the first days but later no fat.

Ligation of a Branch of the Portal Vein Just Before Entrance into the Right Lateral Lobe (*Hunt* [8]).—This operation offers technical difficulties. Despite all precautions, bleeding from the torn vein after the needle is inserted occurs often. Rats in which this took place were not included in this report, although I found that a little bleeding has no significance, the results being the same whether or not bleeding occurs. Rapid and obvious decrease in the size of the right lateral lobe occurred, with no necrosis.

8. Hunt, H. R.: A Laboratory Manual of the Anatomy of the Rat, New York, The Macmillan Company, 1924.

Ligation of a Branch of the Hepatic Artery Just Before Entrance into the Right Lateral Lobe (*Hunt*[8]).—No necrosis resulted. Little or no decrease in the size of the lobes was observed in from one to two weeks.

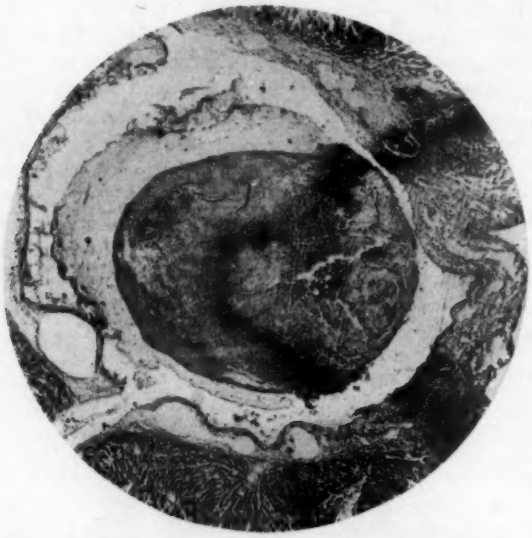

Fig. 2.—Photomicrograph showing thrombosis of a large hepatic vein near the bed of the gallbladder in a rabbit two days after ligation of the hepatic artery.

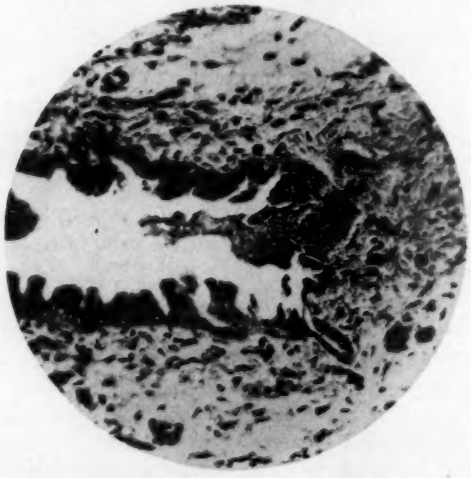

Fig. 3.—Photomicrograph showing partial necrosis of a large bile duct and of the connective tissue surrounding the necrotic area (at the right) in a rabbit, two days after ligation of the hepatic artery.

Ligation of a Branch of the Portal Vein and of the Corresponding Branch of the Hepatic Artery either in Succession or Simultaneously.—Complete necrosis with softening and central liquefaction of one or both of the corresponding lobes resulted.

Ligation of the Main Trunk of the Hepatic Artery and the Branch of the Portal Vein Already Mentioned.—In two of three rats partial necrosis of the central parts of both lobes was observed, and in one of three rats, complete necrosis of both lobes.

Experiments with Injections.—From 0.5 to 1 cc. of a 20 per cent solution of india ink in distilled water was injected into living rats.

1. India ink was injected into the vena cava inferior immediately after ligation of the main trunk of the portal vein. The rats died soon after the injection, and the lungs were observed to be filled with india ink. The liver was either free from ink or slightly black.

2. India ink was injected into a mesenteric vein after ligation of a branch of the portal vein. The lobe supplied by the ligated vein remained free from india ink.

3. India ink was injected into the vena cava inferior after ligation of a branch of the portal vein. The lobe supplied by the ligated branch was obviously less black than the rest of the liver.

4. India ink was injected into the vena cava inferior immediately after ligation of a branch of the portal vein and a branch of the hepatic artery. In two experiments the corresponding lobes of the liver were free from india ink; in one they were slightly black.

5. India ink was injected into the vena cava inferior two days after ligation of the portal vein and a branch of the hepatic artery. The necrotic lobes were free from india ink. Only a narrow zone adjoining the other parts of the liver was black, as was likewise a small portion lying directly on the vena cava (fig. 1).

Ligation of the Hepatic Artery in Rabbits.—Six rabbits died within from one to two days after ligation of the hepatic artery. The liver showed numerous necrotic areas. After ligation of the left branch of the hepatic artery in a rabbit, a second laparotomy revealed no necrosis of the liver. The branch to the right lateral lobe was then ligated, and the animal was killed within twenty-four hours. This lobe was entirely necrotic.

Necrotic Areas in the Liver of Rabbits After Ligation of the Hepatic Artery.—In all the rabbits, as a rule, the gallbladder was observed to be necrotic. A large hepatic vein running along the bed was thrombosed, which was easily seen in transverse sections through the bed of the gallbladder (fig. 2). The necrotic areas within the liver measured from 1 to 3 cm. in diameter and were wedgelike or cuboid. The areas were either dark red and swollen or anemic and gray. In all the areas there were thrombosed vessels, either branches of the portal vein or hepatic veins, with or without adjacent necrotic bile ducts (fig. 3).

COMMENT

Cause of Necrosis of the Liver Following Ligation of the Hepatic Artery.—The ligation of the main trunk of the portal vein or one of its branches or the ligation of the main trunk of the hepatic artery or one of its branches does not produce necrosis in the liver of the rat. To be sure, ligation of a branch of the portal vein resulted in rapid decrease in the size of the liver, and a similar, though less marked, atrophy followed ligation of the hepatic artery, especially in rabbits,

but necrosis does not occur (Rous and Larimore [9]). On the other hand, prompt necrosis invariably results in rats when both a branch of the portal vein and the corresponding branch of the hepatic artery are occluded. That an organ or part of an organ becomes necrotic when it is deprived of its blood supply is an elementary fact. But the results of the present experiments with rats prove much more.

The experiments on rats are of great value in determining the cause of necrosis of the liver which occurs in rabbits, guinea-pigs and human beings after ligation of the hepatic artery. If one considers the results of previous experiments with rabbits [10] and those of recent experiments with rats, especially the observation that only complete obstruction of the blood supply is capable of causing necrosis, it is easy to deduce the cause of the necrosis from the macroscopic and microscopic observations on the necrotic areas of the rabbit liver. The necrotic areas, especially the smaller ones, are similar in size and shape to those of infarcts in the spleen and kidneys. The larger areas are more cuboid, corresponding to the widely spreading and branching character of the hepatic and portal veins. Some are pale and anemic, and others, dark red (in the first hours). Microscopically, the distended capillaries and the hemorrhagic infarction are easily seen. These macroscopic and microscopic features, with the thrombi always seen in branches of the portal vein or in hepatic veins, are apparently sufficient to characterize the areas as infarcts, similar either to anemic or to hemorrhagic infarcts in the heart, spleen, kidneys, etc. In other organs thrombosed (or embolic) vessels and wedgelike necrotic areas, anemic or hemorrhagic, are always considered to be primary and secondary processes, respectively, unless special structures indicate otherwise. There is no reason to interpret the changes in the liver differently.

The reasons for the thrombosis of the portal vein and for that of the hepatic veins may differ. The hepatic artery is the nourishing vessel not only of the gallbladder and bile ducts but of the large branches of the portal vein within the liver so far as they possess a blood supply of their own. The removal of this blood supply should result in necrosis of the wall in the cases in which the blood from the portal vein itself is, for unknown reasons, insufficient to nourish the wall. In a necrotic wall thrombosis is easily possible. For thrombosis in other areas another explanation, based on the primary necrosis of the gallbladder and bile ducts, is possible. The gallbladder is observed to be necrotic as a rule, and the same is true of an area around the bed of the gallbladder measuring about 2 by 2 cm. In this region, a long, large thrombosed

9. Rous, Peyton, and Larimore, L. D.: J. Exper. Med. **31**:609, 1920.

10. Loeffler, L.: Virchows Arch. f. path. Anat. **266**:55, 1927; Arch. f. klin. Chir. **149**:370, 1928

hepatic vein generally runs transversely through the bed, from 2 to 3 mm. from the wall of the gallbladder. One may take it for granted that in this case the bile diffuses from the gallbladder through the necrotic wall and the wall of the vein so that thrombosis is established. The necrosis is either hemorrhagic, due to obstruction of the hepatic veins in the presence of a patent portal vein, or anemic, if the portal vein is obstructed first.

In other parts of the liver one observes necrotic bile ducts encircled partly or completely by a narrow zone of necrotic liver tissue. In such cases the necrosis is evidently caused by diffusing bile. It depends on chance whether the adjacent veins, portal or hepatic, are involved completely, partially or not at all. Thus, the variable appearance, size and color of the necrotic areas are easily explained. Small areas in an early stage become included in large areas in a later stage, while anemic and hemorrhagic and fresh and old infarcts and those of intermediate development are observed together in confusion. As in other organs, thrombi may become larger, and the same thrombus, occlusive in one place, can be seen to be only partially so in other areas; similarly, well preserved bile ducts can be observed within necrotic spots although there is a necrotic portion at the tip of the same area. Necrosis beginning in the central part of the lobe has often been seen and can be explained on this basis. The changes in the margins and the further development of the infarcts need not be described here; the reader is referred to a previous paper.

Reasons for the Maintenance of the Liver After Ligation of the Hepatic Artery.—In the cases in which necrosis occurs after ligation of the hepatic artery or one of its branches, the change is never complete but occurs only in areas, the necrosis usually not comprising the greater part of the liver (Shann and Fradkin[11]). Thus, such livers resemble those in which no necrosis occurs, and the striking difference between the two types is thus diminished. If necrosis is explained on the basis of a completely obstructed blood supply, uinterrupted circulation of the blood would explain the maintenance of the liver. From the clinical point of view it makes no difference which vessel is the remaining source of the blood supply, but theoretically and for the purpose of this investigation it is of great value to determine the source of the blood which maintains the liver after ligation of the hepatic artery, i. e., whether it is the portal vein alone or possible anastomoses as well. Whereas earlier writers thought that the portal vein was the source of nourishment of the liver after ligation of the hepatic artery, later investigators (Harberer, Cameron, Segall[12] and Behrend and his

11. Shann, Hermann, and Fradkin, William: J. A. M. A. **101**:829, 1933.
12. Segall, H. H.: Surg. Gynec. & Obst. **37**:152, 1923.

associates [13]) insisted that besides the obviously extensive portal circulation other sources of nourishment are indispensable. They expressed the belief that arterial blood is necessary for the maintenance of the liver. It has already been mentioned that it may well be questioned whether the hepatic artery conducts arterial blood to the liver. For the present purpose, however, I shall assume that it does.

After ligation of the hepatic artery, arterial blood can be supplied to the liver (*a*) by adding arterial blood artificially and (*b*) by anastomoses. It has been attempted experimentally to replace the arterial blood of the hepatic artery by that of other arteries (Narath [14]).

Other and better sources of an arterial blood supply are natural anastomoses. Anastomoses may originate (*a*) extrahepatically, from branches of the main trunk of the hepatic artery or from the neighborhood of the liver, and (*b*) intrahepatically.

Extrahepatic anastomoses may arise from the right gastric and the gastroduodenal artery, from the phrenic artery and arteries in the ligamentum teres or through adhesions (Hofmeister [15]). There are also often accessory arteries, which arise directly from the aorta or mesenteric arteries (Hecht [16]).

Intrahepatic anastomoses may be formed from connections between the right and the left main branch, from anastomoses between the small arteries within the liver shortly before they become capillaries, from capillary anastomoses and, finally, from subcapsular arteries (Segall [12]).

It is obvious that after ligation of an artery the presence of anastomoses can prevent any effect. On the other hand, one should bear in mind that all the instances in which injections were made after death (Segall [12] and Haberer) only demonstrate the anatomic existence of anastomoses; it was not proved that they are effective enough in any given case. Anastomoses must be sufficiently large and numerous to be effective. Capillary anastomoses and the very small phrenic arteries are too minute to be of any help.

After ligation of a small branch of the artery and of a small branch of the portal vein in rats, necrosis was shown in all instances. After injection of india ink into the inferior vena cava following ligation of these vessels, both lobes were observed to be free from ink. All the supposedly existing anastomoses were not capable of preventing necrosis; only a small margin of the lobe adjoining other lobes remained intact, showing how far intrahepatic anastomoses are efficacious. These exper-

13. Behrend, M.; Radasch, H. E., and Kershner, A. G.: Arch. Surg. **4**:661, 1922.

14. Narath, A.: Deutsche Ztschr. f. Chir. **135**:305, 1916.

15. von Hofmeister, F.: Zentralbl. f. Chir. **48**:154, 1922.

16. Hecht, cited by von Haberer.[1]

iments in rats rendered invalid all attempts to explain the lack of necrosis after ligation of the artery in these animals on the basis of anastomoses, although such an explanation seemed reasonable. If intrahepatic anastomoses were effective, necrosis in rabbits should be less extensive after ligation of a branch only than after ligation of the main trunk. The effect of ligation of the main trunk of the artery has been proved by my experiments and by those of other authors (Cohnheim and Litten [17] and Janson [18]) to be the same as that of ligation of a branch in both rabbits and rats.

The results of the experiments performed do not justify the rejection in every case of the theory that anastomoses are responsible for maintenance, and I do not intend to imply it, but they justify the statement that anastomoses are not the sole factor in the maintenance of the liver. It can be concluded without objection from the results of my experiments on rats and rabbits that the liver can survive on the blood of the portal vein alone. The hepatic artery supplies chiefly the gallbladder and bile ducts. Its ligation sometimes has such terrible sequelae only because the hepatic artery is located within the liver and supplies the branches of the portal vein and bile ducts within the liver. Ligation of the artery is often followed by thrombosis of other patent vessels, such as the portal vein or hepatic veins, so that afterward both vessels are obstructed. This is the real cause of the necrosis. Hence, the question of anastomoses loses its importance.

SUMMARY

Necrosis of liver tissue in rats does not occur unless a branch of the hepatic artery and one of the portal vein are ligated at the same time.

When partial necrosis occurs in any animal after ligation of the hepatic artery, both the hepatic artery and the portal vein may be supposed to be occluded and the blood supply of the corresponding parts completely obstructed. This happens because of thrombosis of branches of the portal vein or of hepatic veins, either directly, through lack of blood supply to the walls of these vessels, or indirectly, owing to necrosis of the wall, caused by extravasation of bile from the necrotic gallbladder or bile ducts in some areas. Obstruction of both vessels in this way results in anemic or hemorrhagic infarcts of the liver.

17. Cohnheim, J., and Litten, M.: Virchows Arch. f. path. Anat. **6**:153, 1876.
18. Janson, C.: Beitr. z. path. Anat. u. z. allg. Path. **17**:505, 1895.

DEVELOPMENT OF SARCOMA IN MALE MICE RECEIVING ESTROGENIC HORMONES

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Certain hydrocarbons that have induced sarcoma in mice when applied to the skin or injected subcutaneously have been stated to possess both estrogenic and carcinogenic activity.[1] These substances are also closely related chemically to the estrogenic hormones.

Mammary carcinoma has been observed in male mice, in which mammary tumors do not normally develop, after repeated injections of estrogenic hormone.[2] Neoplasms of connective tissue have not been observed to arise at the sites of injection following the extended use of pure estrogenic hormones obtained from the urine of pregnant women. When reporting the effect of ovariectomy on mammary carcinoma, Cori [3] mentioned that a spindle cell sarcoma occurred in one male mouse receiving an oil solution of crude estrogenic material. De Jongh and Korteweg [4] observed two sarcomas and nine carcinomas in sixteen castrated male mice bearing ovarian grafts. No tumor was observed

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This investigation has been supported, in part, by grants from the Josiah Macy Jr. Foundation and from the International Cancer Research Foundation.

* This investigation was started while the first author was a National Research Council Fellow. Further support has been given by the Fluid Research Funds of the Yale School of Medicine and by the Committee for Research in Problems of Sex of the National Research Council through grants administered by Professor Edgar Allen.

1. (*a*) Cook, J. W.; Dodds, E. C.; Hewett, C. L., and Lawson, W.: Proc. Roy. Soc., London, s. B **114**:272, 1934. (*b*) Cook, J. W.; Dodds, E. C., and Greenwood, A. W.: Proc. Roy. Soc., London, s. B **114**:286, 1934.

2. Lacassagne, A.: Compt. rend. Acad. d. sc. **195**:630, 1932. Burrows, H.: Am. J. Cancer **24**:613, 1935. Bonser, G. M.: J. Path. & Bact. **41**:217, 1935. Gardner, W. U.; Smith, G. M.; Allen, E., and Strong, L. C.: Arch. Path., to be published.

3. Cori, C. F.: J. M. Research **45**:983, 1927.

4. de Jongh, S. E., and Korteweg, R.: Acta brevia neerl. **5**:126, 1935.

in four control mice. The carcinogenic hydrocarbons, which were extensively studied by Kennaway and his associates,[5] are characterized by their influence on the induction of neoplasms of connective tissue and epithelium.

The present report relates the observation of sarcoma at the site of injection of an estrogenic hormone in five male mice of the Strong C_3H strain. Female mice of this strain show mammary adenocarcinoma at an average age of from 8 to 10 months.[6] Beginning at the ages of 30 or 35 days, theelin [7] in aqueous solution was injected subcutaneously at the rate of 10 rat units daily for sixty-eight days into two mice and for one hundred and two days into three mice. Keto-estrin benzoate [8] in solution in oil was then injected at the rate of 500 international units a week until the mice were killed. The total doses and the periods of treatment are summarized in the table.

Summary of Doses and Periods of Treatment

Mouse	Age at Start, Days	Theelin, Total Rat Units Injected	Keto-Estrin Benzoate		Age at End, Days
			Weeks of Injection	Total International Units Injected	
1	35	1,020	14	7,000	240
2	35	1,020	16	8,000	252
3	35	1,020	17	8,500	261
4	30	680	25	12,500	273
5	30	680	12	6,000	188

A rapidly growing tumor developed at the site of injection under the skin of the back in all the mice of this strain which received the treatment shown in the table. These mice were killed after periods of treatment with theelin and keto-estrin benzoate ranging from one hundred and fifty-eight to two hundred and forty-three days. The tumors were first observed from two to three weeks before autopsy was performed. They all arose in association with one or more of the

5. Kennaway, E. L., and Sampson, B.: J. Path. & Bact. **31**:609, 1928. Cook, J. W.; Dodds, E. C.; Hewett, C. L., and Lawson, W.[1a] Cook, J. W.; Dodds, E. C., and Greenwood, A. W.[1b]

6. Strong, L. C.: Genetics **20**:586, 1935.

7. Dr. O. Kamm, of Parke, Davis & Co., supplied the theelin used in this experiment.

8. The term keto-estrin benzoate used in this paper refers to benzogyno-estryl ($C_6H_5COC(C_{18}H_{21}O)$, which was obtained through Dr. Girard, of Paris, from the Laboratoire Français de Chimiothérapie, at the request of Dr. G. M. Smith. This is presumably the substance that was used by Lacassagne, which he called "folliculin benzoate." The active material was keto-estrin benzoate (3-benzoate-17-keto-1,3,5-estratriene), which was prepared from ketohydroxyestrin derived from the urine of pregnant women. It was obtained in solution in oil (10,000 international units per cubic centimeter).

persisting oil-containing cysts, at a point definitely removed from the mammary glands. On reaching a diameter of from 1 to 2 cc. the tumors threatened to break through the skin and ulcerate. Fragments of the tumors removed at autopsy were grafted into other mice, and the remaining tissue was examined histologically.

All the primary tumors (illustrated by *A* in the figure) and grafts (illustrated by *B*) were spindle cell sarcomas in a state of active growth. Large multinucleated cells were observed occasionally. The cells were usually compactly arranged. Delicate capillaries were plentiful. Edematous areas occurred in the primary tumors, with an associated leukocytic infiltration. There were some areas of necrosis in both the primary tumors and the grafts. The neoplastic tissues showed invasion of the surrounding normal tissues. No metastases were observed.

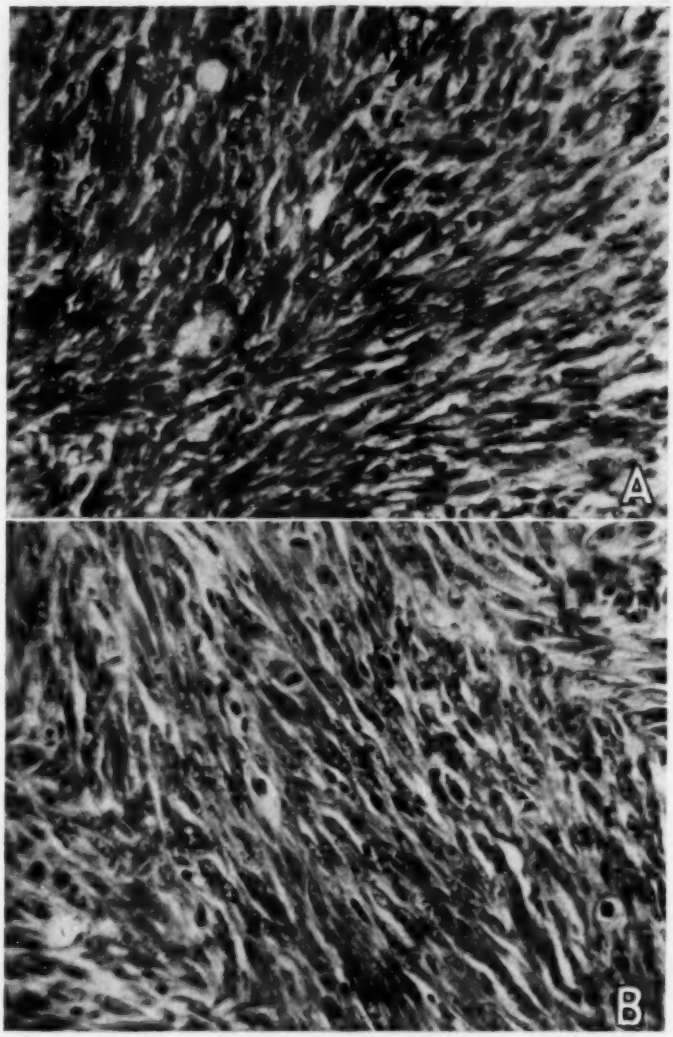
A fragment of each of four of the sarcomas was grafted into each of from one to seven mice of the same strain (C_3H). Only one of the sixteen grafts so made failed to develop.

To the present time all the grafts made in one unrelated strain (JK) have failed to grow. Grafts grew, however, in some of the mice of each of two other distantly related strains and of one unrelated strain. Some of the tissue has been grown as grafts in mice of the C_3H strain for three generations.

Observations have been made on six male mice from another strain (A) which have received identical treatment with the theelin and keto-estrin benzoate. Neither sarcoma nor mammary carcinoma developed in these mice. Six mice of the C_3H strain, which received keto-estrin benzoate for from sixteen to twenty-six weeks, without the previous injection of theelin, have also shown no sarcomas, although the five sarcomas already described developed in mice of this same strain subjected to a different treatment. Though sarcoma may occur spontaneously in mice of the C_3H strain, spontaneous occurrence of this neoplasm has not been observed in either males or females up to the present time in our laboratory.

Allusion should be made to the fact that in all the mice in which a sarcoma developed at the site of injection, it occurred at the margin of an oil-containing cyst in the subcutaneous tissues. Burrows[9] has studied the effects of repeated injections of oil. The subcutaneous connective tissue reacts in an inflammatory manner but does not produce sarcoma. In all our experiments the sarcoma arose in an area away from mammary tissue. The mammary tissue, however, showed the usual pattern of growth following repeated injections of the estrogenic hormone. The specific response of the mammary epithelium to

9. Burrows, H.: Proc. Roy. Soc., London, s. B **111**:238, 1932.



A, photomicrograph of a primary spindle cell sarcoma arising in relation to a subcutaneous oil-containing cyst in mouse 1. This mouse had received a total of 1,020 rat units of theelin and 7,000 international units of keto-estrin benzoate dissolved in oil. Magnification, 300 ×. *B*, photomicrograph of one of the grafts of the sarcoma which had arisen in mouse 5. Magnification, 300 ×.

estrogenic hormone is now generally recognized. There may be associated with this a slight increase in interacinar connective tissue. The development of sarcoma in subcutaneous tissue, however, suggests the possibility that estrogenic hormone, under experimental conditions as yet little understood, may have a stimulating effect on mesodermal cells as well as on epithelium, to the extent of causing neoplasms, as is characteristic of the so-called carcinogenic chemicals.

SUMMARY

Spindle cell sarcomas developed in all of five male mice from two different litters of the C_3H strain which had received subcutaneous injections of 10 rat units of theelin daily for from sixty-eight to one hundred and two days, followed by weekly injections of 500 international units of keto-estrin benzoate for periods of from twelve to twenty-five weeks. These sarcomas grew rapidly in the original mice and also following implantation into other mice of the same strain.

REACTIVITY OF MALIGNANT NEOPLASMS TO BACTERIAL FILTRATES

II. RELATION OF MORTALITY TO HEMORRHAGIC NECROSIS AND REGRESSION ELICITED BY CERTAIN BACTERIAL FILTRATES

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As described in a previous report,[1] bacterial factors capable of eliciting the phenomenon of local skin reactivity in rabbits also produce, on intravenous injection, hemorrhagic necrosis and regression of rapidly growing transplantable malignant tumors in guinea-pigs, mice and rats. Death occurs in a high percentage of the animals, however, shortly after the injection.

My purpose in the experiments reported in this paper was to determine whether there exists any relation between the destruction of the tumors and the mortality of the animals, and also to determine whether the lethal effect could be reduced without impeding the tumor-destructive potency of the preparations.

EXPERIMENTS

The tumor employed was mouse sarcoma 180 described in detail in a previous report.[1] The types of bacteria employed were Meningococcus group III (44B); Bacillus typhosus (strain T_1); Bacillus enteritidis (a rough strain of low virulence described in the previous report [3H][1]); Streptococcus haemolyticus (strain 21772, isolated from a case of pharyngitis and of high virulence for mice); Bacillus tuberculosis of human type (strain 4777 of the American Type Culture Collection); Bacillus coli (laboratory stock strain), and Staphylococcus aureus (a strain isolated from a tumor-bearing mouse). The filtrates of these strains of various micro-organisms were as follows: "Agar washings" filtrates of Meningococcus, B. enteritidis, B. coli, B. typhosus and Staph. aureus; a filtrate of Str. haemolyticus obtained from a two day old culture in 0.4 per cent dextrose broth, p_H 7.4, and a filtrate of B. tuberculosis obtained from a two and a half month old culture in 5 per cent glycerin broth. The ability of the preparations to elicit the phenomenon

From the Laboratories of the Mount Sinai Hospital.

This investigation has been aided by a grant from the Josiah Macy Jr. Foundation.

1. Shwartzman, G.: Reactivity of Malignant Neoplasms to Bacterial Filtrates: I. Effect of Spontaneous and Induced Infections on the Growth of Mouse Sarcoma 180, Arch. Path., **21**:284 (March) 1936.

of local skin reactivity was determined in rabbits shortly before use. The reacting titers of the filtrates are recorded in table 1.

The experiments described in this paper were carried out in mice bearing twelve day old tumors. The injections were given either intravenously or intraperitoneally. The gross and microscopic effects of an active preparation on the tumors are illustrated in figures 1 to 5.

As may be seen, sarcoma 180 was already markedly hemorrhagic four hours after the intravenous injection. In many instances the hemorrhage extended farther twenty-four hours later. Beginning with the second day following the injection, there was formation of a dry hemorrhagic scab with a surrounding zone of apparently active growth. About the sixth or seventh day, the scab fell off, leaving a bed of granulation tissue which, in many instances, became completely healed. It is of considerable interest that the zone of actively proliferating tumor

TABLE 1.—*Reacting Potency of Bacterial Filtrates Employed*

Filtrate	Micro-Organism	Reacting Units per Cc.
T. 1937	Meningococcus, group III (44B)	4,000
T. 1968	Meningococcus, group III (44B)	3,000
T. 1938	B. typhosus T_1	600
T. 1986	B. typhosus T_1	500
T. 1990	B. enteritidis (rough 3H)	500
T. 1964	B. coli	500
T. 1941	Str. haemolyticus (strain 21772)	5
T. 1954	B. tuberculosis (human strain)	±

tissue seen the first few days after the intravenous injection might unexpectedly undergo complete regression within the second week following the injection. This course of events is also illustrated by histologic sections: The reaction four hours after the injection was that of an abundant hemorrhage into the tumor tissue with disruption of the tumor. Most of the tumor cells, however, were viable. Twenty-four hours after the injection, there appeared extensive necrosis of the tumor. This necrotic zone in the following few days began to be sharply delimited from zones of active growth. Approximately one week after the intravenous injection, there was a small residual mass of tumor undergoing necrosis and regression. Approximately three weeks after injection of the filtrate, the healed site showed a small area of granulation tissue. No tumor cells could be discovered microscopically.

Hemorrhage appearing throughout the entire tumor within twenty-four hours following the injection was noted as a 4+ reaction. This reaction was obtained with potent preparations injected intravenously or intraperitoneally in sufficiently large doses. A reaction of this type was followed, in most instances, by complete regression provided the animals

survived for a sufficiently long period of time. Thus far, completely healed tumors never resumed growth and showed no metastases in mice observed for periods as long as from four to five months after the intravenous injection. It is not certain, however, whether sarcoma 180,

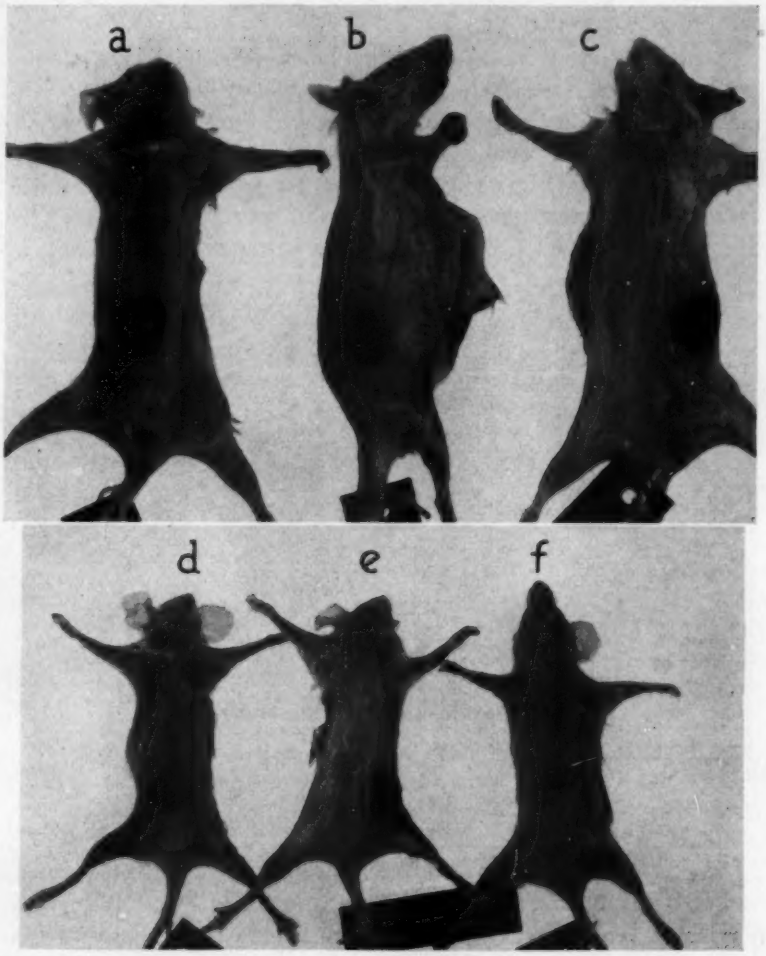

Fig. 1.—Mice bearing twelve day old tumors received each 100 units of B. typhosus "agar washings" filtrate T.1937 intravenously. Note the severe hemorrhage in the tumor tissue four hours later (*a* and *b*); and twenty-four hours later (*c*); the dry hemorrhagic crust two days later (*d*) and four days later (*e*), and the healing of the lesion eight days later (*f*). (In *a* the tumor is cross-sectioned.)

employed in this work, is prone to give rise to metastases. Incomplete hemorrhages noted as 1+, 2+ and 3+ reactions were followed by

partial regressions. The edges of the tumor in some of the cases resumed growth during the period of from two to four weeks following the injection.

Table 2 presents a summary of the effects of the various bacterial filtrates on the growth of sarcoma 180 and the mortality associated with the use of these preparations.

As may be seen in table 2, the meningococcus "agar washings" filtrates contained a tumor-destructive principle of high potency. Prompt hemorrhages leading to complete regression of the tumors in most of the surviving mice were obtained consistently by intravenous and intraperitoneal injections. The subcutaneous [2] injections produced hemorrhages of similar severity. A few observations seem to suggest that the incidence of regressions of tumors may be lower in mice thus treated.

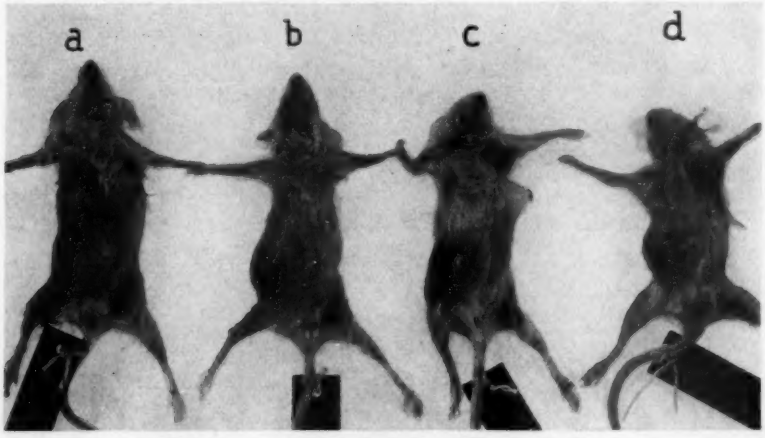

Fig. 2.—Control series of mice. Note the twelve day old tumor (*a*); the twelve day old tumor cross-sectioned (*b*); the fifteen day old tumor (*c*), and the thirty-five day old tumor (*d*).

Meningococcus "agar washings" filtrates injected intravenously in doses ranging from 750 to 3,000 reacting units killed twenty-six of twenty-nine tumor-bearing mice within twenty-four hours (groups 1 to 4). Intraperitoneal injections of these filtrates in doses of 1,000 and 2,000 reacting units killed nine of the thirteen tumor-bearing mice (groups 5 and 6) within twenty-four hours. From two to twenty days later, six more mice from groups 1 to 6 died. Thus, only one of the forty-two tumor-bearing mice survived the intravenous and intraperitoneal injections.

2. The subcutaneous injections were always given at a distance not less than 1 inch (2.5 cm.) from the site of the tumor.

TABLE 2.—*Effects of Bacterial Filtrates on Growth of Sarcoma 180*

Group	Mice	Appearance and Size of Tumor Prior to Treatment	Material Injected¶	Units for Each Injection	Route of Injection	Mortality		Effect		Survivors	
						At 24 Hours	At 2-20 Days	Hemorrhage 48 Hours Following Injection	Regression 2-20 Days Following Injection	Mice	Regression
1	8	Gr.* ++++/4,† +++/3	Mg.44B.T1937.....	3,000	Intravenous	8	0	++++/7†	.....	0	.....
2	7	Gr. +++/7	Mg.44B.T1937.....	1,500	Intravenous	5	1	++++/5 ++/2	Complete/1†	1	Complete/1†
3	10	Gr. +/3, ++/7	Mg.44B.T1908.....	1,000	Intravenous	10	0	++++/5	.....	0	.....
4	4	Gr. ++/3, +++/1 Hem.* +++/1	Mg.44B.T1937.....	750	Intravenous	3	1	++++/4	Complete/1	0	.....
5	3	Gr. +++/3	Mg.44B.T1908.....	2,000	Intraperitoneal	3	0	++++/3	.....	0	.....
6	10	Gr. +/5, ++/5	Mg.44B.T1908.....	1,000	Intraperitoneal	6	4	++++/6 ++/4	Complete/3, incomplete/1	0	.....
7	8	Gr. +/5, ++/2, ++++/1	Mg.44B.T1937.....	3,000	Subcutaneous	5	3	++++/6	Complete/2, absent/1	0	.....
8	13	Gr. ++/13	Mg.44B.T1937.....	3,000§	Subcutaneous	0	7	++++/7	.....	6	Complete/3, absent/3
9	5	Gr. ++/3, +++/2	Mg.44B.T1937.....	1,500	Subcutaneous	5	0	+++/2	.....	0	.....
10	6	Gr. +++/6	Mg.44B.T1937.....	750	Subcutaneous	2	3	++++/6	Complete/1, incomplete/2	1	Complete/1
11	10	Gr. +/6, ++/4	B. enteritidis (rough strain) T1990	500	Intravenous	10	0	++++/2 +++/6	.....	0	.....
12	5	Gr. +++/4, ++/1	B. enteritidis (rough strain) T1990	250	Intraperitoneal	5	0	++++/2 +++/3	.....	0	.....
13	9	Gr. +/5, ++/4	B. enteritidis (rough strain) T1990	125	Intraperitoneal	4	1	++++/4	Complete/2	4	Complete/4
14	4	Gr. +/4	B. coli T.1964.....	250	Intravenous	4	0	+/1	.....	0	.....
15	7	Gr. +/2, ++/5	Str. haemolyticus strain 21772. T.1941	?	Intravenous	4	2	++/1 +/1	.....	1	Incomplete/1
16	3	Gr. ++/2, +/1	Str. haemolyticus strain 21772. T.1941	?	Intraperitoneal	0	3	++++/3	Complete/2, incomplete/1	0	.....
17	4	Gr. ++/2, +/2	Human B. tuberculosis T1954	.....	Intravenous	2	1	++++/2	Complete/1, incomplete/1	1	Complete/1
18	7	Gr. +/4, ++/3	Staph. aureus filtrate	.....	Intravenous	1	1	.....	.....	5	.....
19	5	Gr. +/2, ++/3	TyT_1 ¶ T.1938.....	250	Intravenous	5	0	++++/4	.....	0	.....
20	5	Gr. +++/5	TyT_1 T.1938.....	125	Intravenous	4	0	+++/3	.....	1	Complete/1
21	5	Gr. +/2, ++/3	TyT_1 T.1938.....	250	Intraperitoneal	4	1	+++/1	Complete/1	0	.....
22	5	Gr. +++/4, ++/1	TyT_1 T.1938.....	125	Intraperitoneal	3	1	++++/4	.....	1	Complete/1
23	7	Gr. +/7	Formaldehydized TyT_1.T.1938	250	Intravenous	7	0	++++/6	.....	0	.....
24	3	Gr. +/3	Formaldehydized TyT_1.T.1938	250	Intraperitoneal	3	0	++++/3	.....	0	.....

* Gr. means growth; Hem., hemorrhage.
† Denominators indicate number of mice.
§ In two doses of 1,500 units each within forty-eight hours.
¶ Mg.44B. designates "agar washings" filtrates of Meningococcus group III cultures; TyT_1, "agar washings" filtrates of B. typhosus strain T_1 cultures.
? Not determined.

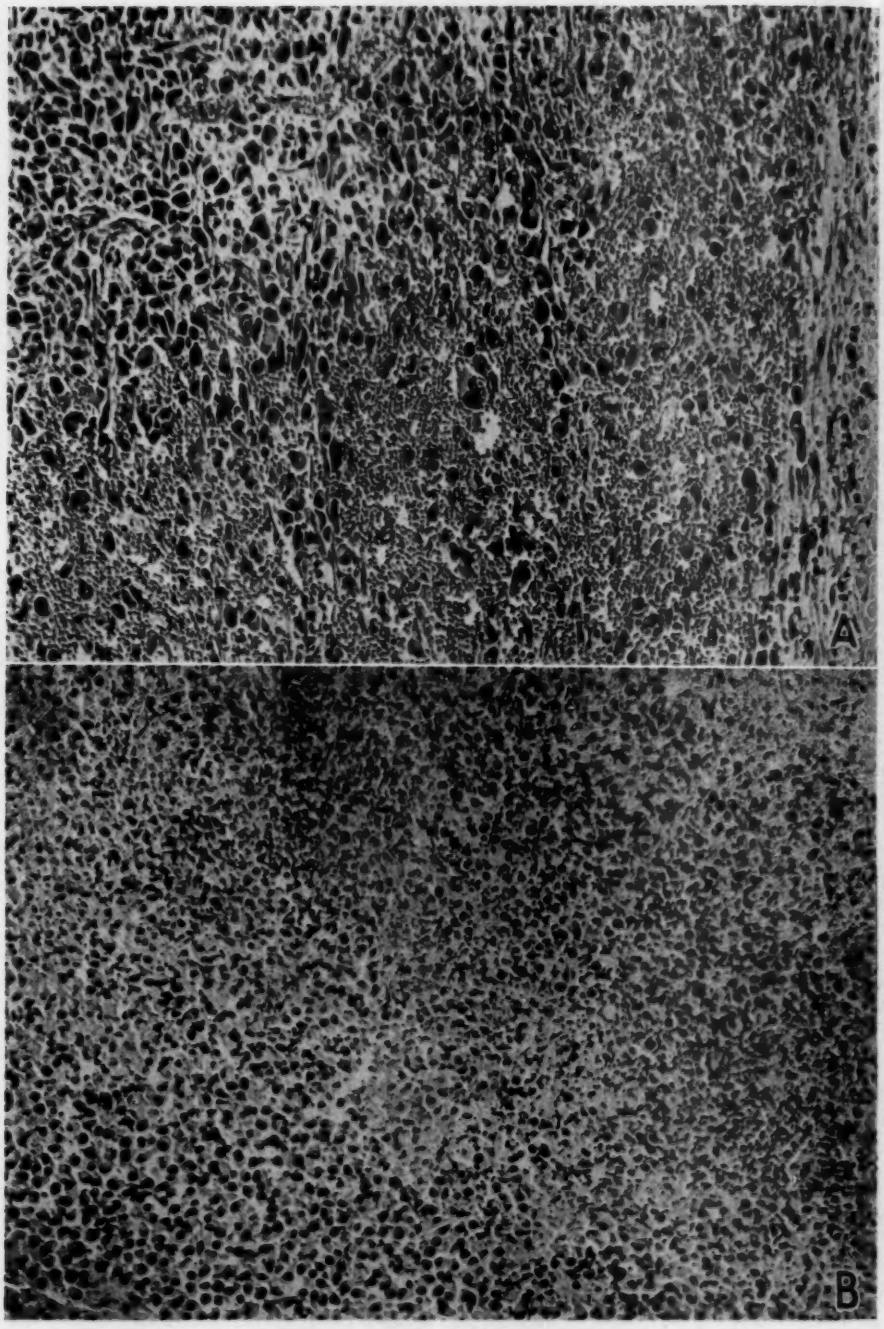

Fig. 3.—Sections of tumor; × 220. A mouse bearing a twelve day old tumor received 100 units of B. typhosus "agar washings" filtrate T.1937 intravenously. *A* shows the histologic appearance of the tumor four hours after the injection. Note the severe hemorrhage. Many viable cells are present. *B* shows the histologic appearance twenty-four hours after the injection. Note the extensive necrosis and hemorrhage.

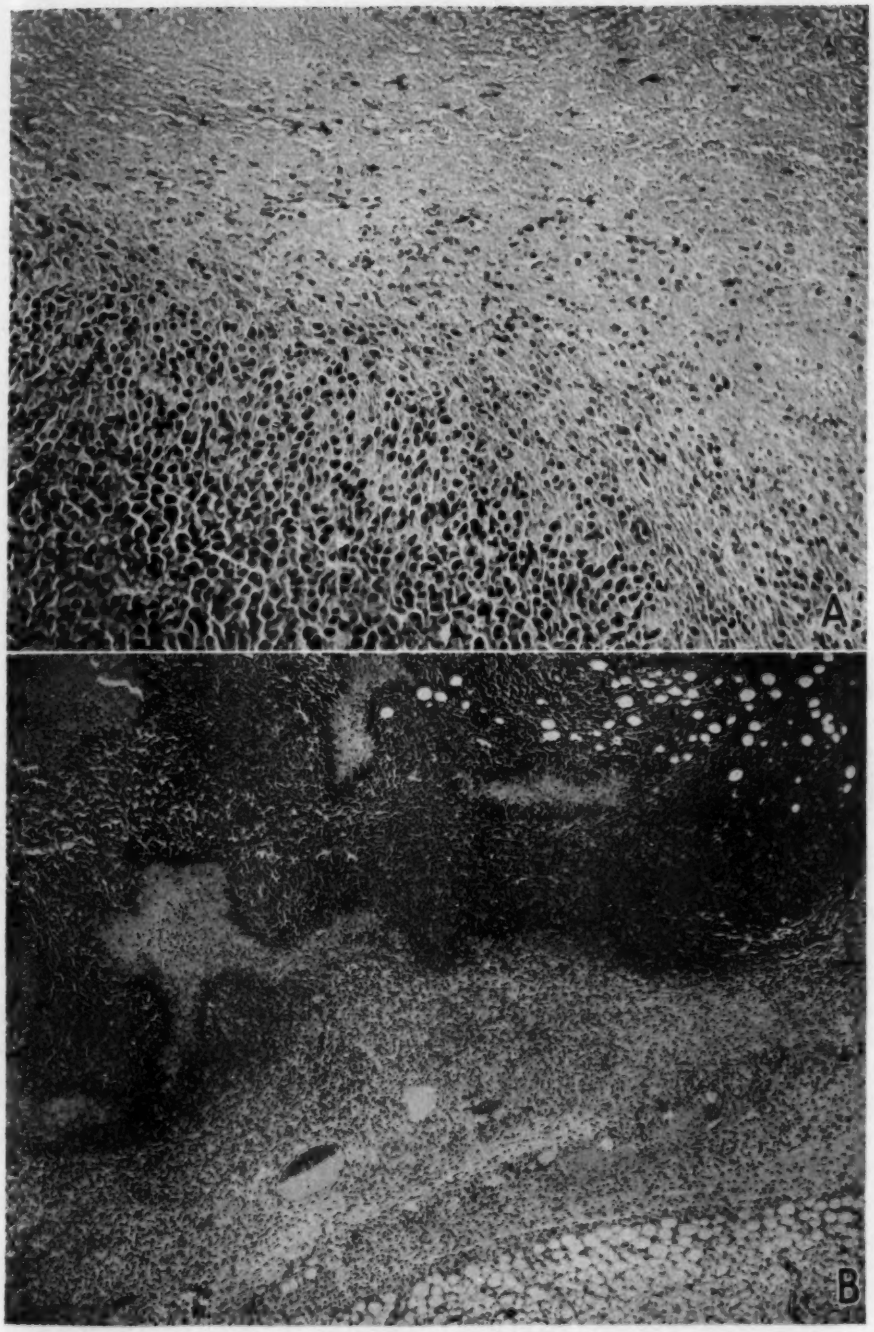

Fig. 4.—Sections of tumor. A mouse bearing a twelve day old tumor received 100 units of B. typhosus "agar washings" filtrate T.1937 intravenously. *A* (× 220) shows the histologic appearance of the tumor forty-eight hours after the injection. Note the wide zone of necrosis demarcating the residual tumor. *B* (× 50) shows the histologic appearance eight days after the injection. Note the small residual mass of tumor undergoing necrosis and regression.

As may be seen in table 3, the lethal effect of meningococcus filtrates on normal mice was considerably lower. Of thirty-seven mice given intravenous and intraperitoneal injections of meningococcus filtrates in doses of approximately similar range, only nineteen died (groups 1 to 10). Here again the highest mortality was observed within the first twenty-four hours.

A comparison of intravenous and intraperitoneal injections with respect to toxicity brings out a curious fact. Intravenous and intra-

TABLE 3.—*Effects of Various Bacterial Filtrates on Normal Mice*

Group	Mice	Material Injected*	Units for Each Injection	Route of Injection	Mortality		
					At 24 Hours	At 2-20 Days	Survivors
1	4	Mg.44B.T1937.....	3,000	Intravenous	2	0	2
2	4	Mg.44B.T1937.....	2,700	Intravenous	4	0	0
3	8	Mg.44B.T1968.....	2,000	Intravenous	0	1	7
4	3	Mg.44B.T1968.....	1,500	Intravenous	1	0	2
5	4	Mg.44B.T1937.....	1,500	Intravenous	1	0	3
6	3	Mg.44B.T1937.....	750	Intravenous	1	0	2
7	3	Mg.44B.T1913.....	3,000	Intraperitoneal	3	0	0
8	3	Mg.44B.T1968.....	3,000	Intraperitoneal	2	0	1
9	2	Mg.44B.T1968.....	2,000	Intraperitoneal	0	1	1
10	3	Mg.44B.T1968.....	1,500	Intraperitoneal	3	0	0
11	7	Mg.44B.T1968.....	4,000	Subcutaneous	2	3	2
12	9	B. coli T.1964.....	.....	Intraperitoneal	4	1	4
13	5	B. enteritidis T.1990..	125	Intraperitoneal	2	0	3
14	5	B. enteritidis T.1990..	250	Intraperitoneal	5	0	0
15	5	TyT$_1$ T1938.....	125	Intravenous	1	0	4
16	5	TyT$_1$ T1938.....	125	Intraperitoneal	0	2	3
17	5	TyT$_1$ T1938.....	250	Intravenous	5	0	0
18	5	TyT$_1$ T1938.....	250	Intraperitoneal	3	1	1
19	4	TyT$_1$ T1938 +.....	200	Intravenous	0	0	4
		H.682.....	200				
20	4	TyT$_1$ T1938 +.....	500	Intravenous	1	1	2
		H.682.....	200				
21	4	TyT$_1$ T1938+.....	500	Intraperitoneal	2	1	1
		H.682.....	200				
		Supernatant fluid mixture of:					
22	4	TyT$_1$ T1938 +.....	750	Intravenous	2	0	2
		H.682.....	200				

* Mg.44B. designates "agar washings" filtrates of Meningococcus group III cultures; TyT$_1$, "agar washings" filtrates of B. typhosis strain T$_1$ cultures; H.682, antityphoid serum containing 800 neutralizing units per cubic centimeter.

peritoneal injections are of equal toxicity to tumor-bearing mice. Thus, all of thirteen tumor-bearing mice given intraperitoneal injections and twenty-eight of twenty-nine tumor-bearing mice given intravenous injections were killed. To normal mice however, intravenous injections, appear to be decidedly less toxic than intraperitoneal injections. Thus, death occurred in nine of twenty-three normal mice given intravenous injections of doses ranging from 1,500 to 3,000 reacting units (groups 1 to 5, table 3) and in nine of eleven normal mice given intraperitoneal injections of the same doses (groups 7 to 10, table 3).

Subcutaneous injections of meningococcus "agar washings" filtrates in doses ranging from 750 to 3,000 reacting units are less toxic than intraperitoneal and intravenous injections to tumor-bearing mice. In this series, seven of thirty-two tumor-bearing mice survived (groups 7 to 10, table 2). As will be remembered, similar doses injected intravenously and intraperitoneally killed forty-one of forty-two tumor-bearing mice (groups 1 to 6, table 2). As may be seen in table 2, there is a certain irregularity in the lethal effect of subcutaneous injections of the filtrates on tumor-bearing mice. Thus, in group 7, the subcutaneous

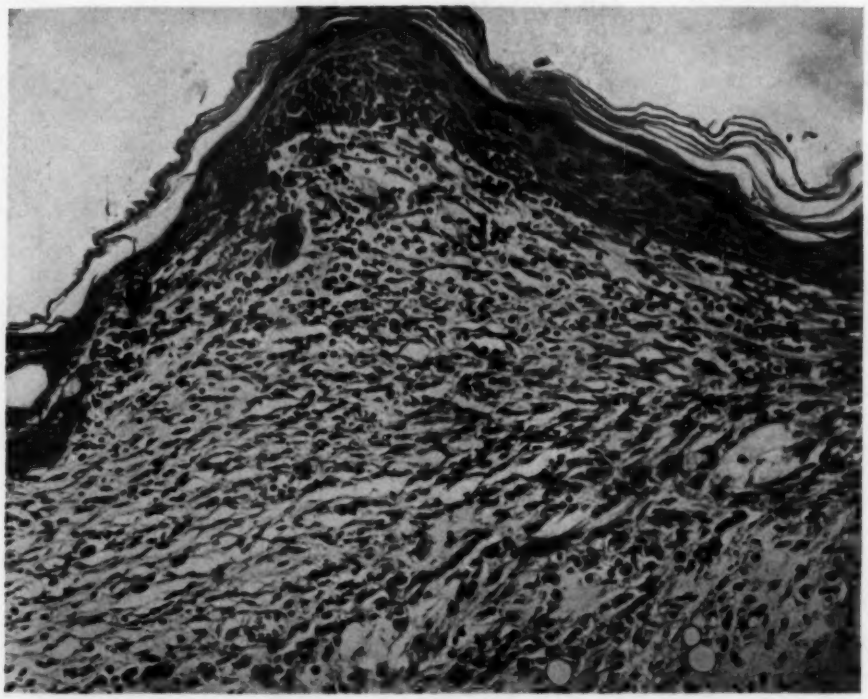

Fig. 5.—Section of tumor; × 220. A mouse bearing a twelve day old tumor received 100 units of B. typhosus "agar washings" filtrate T.1937 intravenously. The section shows the histologic appearance nineteen days after the injection. Note the area of granulation tissue. No tumor cells are seen.

injection of 3,000 reacting units killed five mice twenty-four hours later and three mice from two to twenty days later, and in group 9 the subcutaneous injection of 1,500 reacting units killed all the mice tested. In another instance (group 8), when 3,000 reacting units was administered in two doses of 1,500 reacting units twenty-four hours apart, the first injection produced no effect, and seven of thirteen mice died twenty-four hours following the second injection.

No comparison of the lethal effects of subcutaneous injections on normal and tumor-bearing mice is attempted, since the number of normal mice employed was small.

B. typhosus "agar washings" filtrates also showed a decidedly higher toxicity for tumor-bearing mice, the greatest mortality being elicited within twenty-four hours. Two of thirty tumor-bearing mice (groups 19 to 24, table 2) and eight of twenty normal mice (groups 15 to 18, table 3) survived intravenous and intraperitoneal injections of doses ranging from 125 to 250 reacting units.

B. enteriditis culture filtrates in the doses employed were equally toxic to normal and tumor-bearing mice. Doses of 125 to 250 reacting units injected intravenously and intraperitoneally killed all the mice tested within twenty-four hours.

As may also be seen in table 2, B. coli, Str. haemolyticus, B. tuberculosis (human strain) and Staph. aureus culture filtrates injected intravenously and intraperitoneally killed a high proportion of mice. Of these filtrates, the Staph. aureus produced no effect and the Str. haemolyticus, B. tuberculosis and B. coli only slight effects on the tumor. The latter experiments, although carried out with an inadequate number of mice, suggested that the tumor-destructive potency of bacterial filtrates is independent of their lethal effect.

In the course of certain neutralization experiments, the observation was frequently made that in certain proportions mixtures of B. typhosus filtrates with homologous antiserums were completely devoid of lethal effect and yet retained their power of eliciting hemorrhagic necrosis in the skin of a high percentage of prepared rabbits. This may be illustrated by the following example:

Twenty-five reacting units of B. typhosus "agar washings" filtrate T.1938 injected intravenously killed approximately 90 per cent of rabbits tested; 200 reacting units of the same filtrate mixed with 200 antitoxic units of horse serum H.682 gave no reactions in prepared rabbits and had no lethal effect; a mixture of 500 reacting units of this filtrate with 200 antitoxic units of the horse serum elicited severe hemorrhagic and necrotic reactions in eight of ten rabbits tested but had no lethal effect; a mixture of 750 reacting units of the filtrate with 200 antitoxic units of the horse serum produced reactions in seven and killed two of eight rabbits tested.

In view of the foregoing facts, it was of interest to study the effects of mixtures of B. typhosus filtrates and homologous antiserums in various proportions on normal and tumor-bearing mice.

From the small series of experiments of groups 19 to 22 of table 3, it may be seen that a decided protection is afforded in normal mice by the antiserums inasmuch as 200 reacting units of B. typhosus filtrate mixed with 200 units of antitoxin H.682 produced no effect when

injected intravenously. A mixture of 500 reacting units of the filtrate plus 200 units of the serum killed two of four mice while it failed to kill any rabbits. It may appear, therefore, that the mixtures are more toxic to normal mice than to rabbits. This, however, remains questionable because the total amount per weight was much larger in mice than in rabbits (i. e., 1.25 cc. per kilogram of body weight of rabbits and 1 cc. per each mouse).

Figure 6 represents results obtained with B. typhosus "agar washings" filtrates and mixtures thereof with homologous neutralizing horse

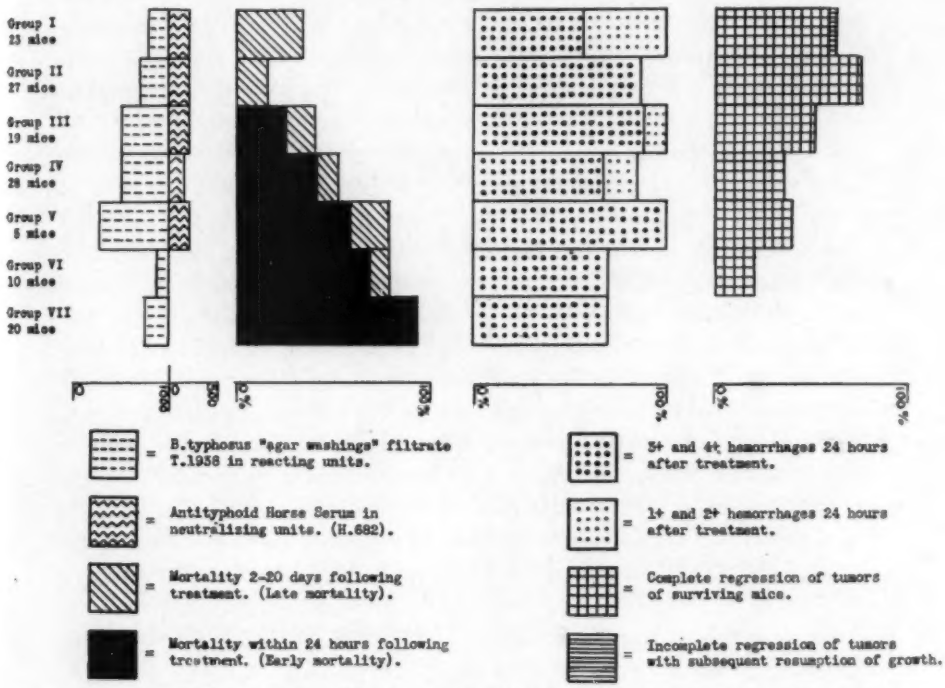

Fig. 6.—Effects of B. typhosus toxin and mixtures of such toxin with antityphoid horse serums on mouse sarcoma 180.

serum (H. 682) in 132 tumor-bearing mice. Seventy-nine mice were given intravenous and fifty-three mice intraperitoneal injections. Inasmuch as no appreciable difference was obtained in mice treated by these routes, the figures were added together for simplicity of presentation.

As may be seen in figure 6, the serum afforded unquestionable protection against the lethal effect of the filtrates. With certain amounts no mortality was obtained within the first twenty-four hours and only a low mortality within from two to twenty days following the injection

(groups 1 and 2). As the amount of toxin in the mixture was increased, the mortality was proportionately raised (groups 3 to 5). A discrepancy was observed, however, in groups 1 and 2 in which a higher late mortality occurred with 200 reacting units mixed with 200 antitoxic units of serum than with 300 reacting units mixed with 200 antitoxic units of serum. The difference, although not very striking, is possibly explained as a prozone reaction.

It also becomes obvious in figure 6 that hemorrhage, necrosis and subsequent complete regression may be obtained in a large proportion of tumors with partially neutralized toxic filtrates of low lethal potency. Thus, one combination (group 2) showed no early mortality (i. e., within the first twenty-four hours), a low late mortality and a strikingly high proportion of severe reactions in the tumor tissue and of complete regressions. The incidence of hemorrhagic necrosis continued to be high as the number of toxic units in mixture with the serum was raised. There was a somewhat lower incidence of hemorrhage and necrosis in mice treated with toxin alone. This, possibly, was due to the fact that some mice died too early. An assumption of this sort may be made safely since some of the reactions require fully twenty-four hours for their appearance. It may also be seen in figure 6 that the hemorrhage and necrosis were followed by complete regression in a high proportion of animals. Many of the surviving mice with complete regression were kept for periods as long as from four to five months. No resumption of growth or metastases was observed in these mice. From the analysis of the column indicating the proportion of tumor regression, it may appear that the incidence of regression decreased as the number of toxic units increased. This conclusion would be erroneous, however, since many of the mice died within the first twenty-four hours.

Thus, the experiments recorded in figure 6 prove definitely that the ability of a given bacterial filtrate to elicit hemorrhage, necrosis and regression of sarcoma 180 is a function independent of its lethal effect. In previous experiments it was shown that certain bacterial filtrates were more toxic to tumor-bearing mice than to normal mice. It appears, then, that the higher death rate was not due to the processes elicited in the tumor. It is well known that many tumor-bearing mice have secondary infections as early as twelve days after transplantation. It was thought that the high lethal effect of bacterial filtrates on tumor-bearing mice might be in the nature of an anaphylactoid or a hypersensitive reaction made possible by sensitization with secondary invaders. The following experiment was done in order to study this point:

Four normal mice (group 1) and three mice bearing twelve day old tumors (group 2) were each given an intravenous injection of 1 cc. of an eighteen hour old broth culture of a rough strain of B. enteritidis

(3H) diluted 1 : 100 in 0.85 per cent sodium chloride solution. The low virulence of the strain in this dilution was previously determined.[1] Five days later these mice and four normal mice (group 3) received each intravenously 200 reacting units of meningococcus "agar washings" filtrate. Three mice of group 1 and all the mice of group 2 died within twenty-four hours, and three mice in group 3 died five days after the intravenous injection of meningococcus filtrate. The remaining mice of groups 1 and 3 survived.

As may be seen from this experiment, the injection of 200 reacting units of meningococcus "agar washings" filtrate elicited death within twenty hours in most of the mice infected with a strain of B. enteritidis of low virulence. Normal mice resisted this injection for five days. It is suggestive, therefore, that the secondary infections of tumor-bearing mice may render them more susceptible to the lethal effect of the bacterial filtrates employed in this work.

COMMENT AND CONCLUSIONS

In this paper are embodied experiments concerning the relationship between the hemorrhagic necrosis of sarcoma 180 in mice and the mortality of the mice elicited by injections of certain bacterial filtrates.

Comparative studies on the lethal effects of bacterial filtrates on normal mice and mice bearing twelve day old sarcoma 180 were made. It appeared that the preparations employed were decidedly more toxic to tumor-bearing than to normal mice. The hemorrhagic and necrotic reactions in the tumor tissue could not be held directly responsible for the high death rate. This assumption was suggested first by the fact that preparations lacking in the tumor-destructive principle or those possessing a low concentration thereof also displayed a high toxicity for tumor-bearing mice. Attempts were then made to obtain preparations of high tumor-destructive but low lethal potency.

In the course of the work on the phenomenon of local skin reactivity to bacterial filtrates in rabbits the observation was frequently made that mixtures of B. typhosus "agar washings" filtrates with homologous neutralizing horse serums in certain proportions were completely devoid of lethal potency and yet retained their power of eliciting hemorrhagic necrosis in a high percentage of prepared rabbits. As it was shown previously that the tumor-destructive factors were closely related to or identical with those necessary for the elicitation of local skin reactivity in rabbits, it was decided to test these mixtures of B. typhosus "agar washings" filtrates with antiserums on normal and tumor-bearing mice. It appears from these experiments that the antiserums afford decided protection against the lethal effect of B. typhosus "agar washings" filtrate on normal and tumor-bearing mice. In certain proportions,

mixtures of B. typhosus "agar washings" filtrate with antiserums produce no early mortality (i. e., within twenty-four hours following the injection) and a low rate of late mortality and yet are capable of eliciting prompt and intense hemorrhagic necrosis with subsequent complete regression of sarcoma 180 in a high percentage of mice. This incidence of regression may be considered well above the normal expectancy (i. e., group 2, figure 6, regression in twenty-one of twenty-three surviving mice) since usually only 1.33 per cent spontaneous regressions of this tumor occur.[1] As the amount of filtrate in mixture with the same amount of serum is increased, there occur early mortality and a roughly proportionate rise in late mortality. The incidence of complete regressions of tumors in surviving mice remains then approximately the same. Doses of 125 and 250 reacting units of B. typhosus "agar washings" filtrate alone (i. e., without the serum) elicit early mortality in as many as 70 and 95 per cent of mice, respectively. The experiments prove that the tumor-destructive potency is a function independent of the lethal effect of bacterial filtrates. Further attempts to reduce the lethal effect of these and other filtrates by various methods are under way.

It appears, therefore, from the observations described, that the process of tumor destruction is not necessarily directly responsible for a higher death rate in tumor-bearing mice than in normal mice treated with the bacterial filtrates. Further evidence of this fact was afforded in some experiments in which normal and tumor-bearing mice were given injections of a strain of B. enteritidis of low virulence. In the normal and tumor-bearing mice thus infected, equally high death rates were obtained following the intravenous injections of a small dose of meningococcus filtrate. These experiments suggest that incidental secondary infections of tumor-bearing mice may sensitize them to the point of causing anaphylactoid reactions to bacterial filtrates.

Incidentally, attention should be drawn to the fact that a zone of actively growing neoplastic tissue may be observed at the periphery of a hemorrhagic tumor within the first week following the intravenous injection of an active preparation. The zone may later unexpectedly undergo complete regression. This observation was previously made by Apitz [3] and attributed by him to the development of Caspari's "necrohormones" subsequent to the hemorrhagic reaction in the tumor. Experiments on the nature of this reaction are under way.

SUMMARY

There is observed a decidedly higher death rate following injections of bacterial filtrates in tumor-bearing mice as compared with normal mice. Attention is drawn to the possible rôle which sensitization by

3. Apitz, K.: Ztschr. f. Krebsforsch. **40**:50, 1933.

secondary bacterial invaders may play in the production of this high death rate. The process of tumor destruction is not necessarily directly responsible for the high death rate.

In certain proportions, mixtures of B. typhosus "agar washings" filtrate with homologous antiserum possess a comparatively low lethal potency and yet elicit prompt and intense hemorrhagic necrosis and subsequent complete regression of sarcoma 180 in a high percentage of mice well above the normal expectancy.

Case Reports

NEURO-EPITHELIAL CYST OF THE THIRD VENTRICLE

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The neuro-epithelial cyst, sometimes less correctly called the colloid cyst, is a benign primary tumor of the third ventricle of the brain. Only fifty-two authentic cases have been hitherto described.

This paper reports clinical and postmortem information on three additional cases. Adequate reviews of the literature and discussions of the various aspects of this condition have been published by Zimmerman and German,[1] Stookey[2] and Dandy.[3] Additional cases have been reported by Masson,[4] Jones and Collins,[5] Eskelund[6] and Paterson and Leslie.[7]

REPORT OF CASES

CASE 1.—A white Hungarian woman of 43 years was admitted to the Lakeside Hospital on Aug. 16, 1927. Six days before admission she had remained home from work because of a severe headache which, she said, was relieved by medicine prescribed by a physician. She worked every subsequent morning, including the morning of the day of admission. Her husband reported that at noon on that day she appeared normal, but that at 5 p. m. she was found lying on the floor unconscious. She recovered sufficiently to mutter a few words and was then seized with a generalized clonic convulsion lasting for three minutes. She never regained consciousness thereafter. No previous history of significance was obtainable.

She was a moderately well nourished, middle-aged white woman, unconscious and breathing slowly, irregularly and deeply but without apparent effort. The pupils measured 4 mm. in diameter and were equal and regular but fixed to light. The eyelids were two-thirds closed and symmetrical. There was no strabismus, nystagmus or migratory movement of the globes. The fundi showed no hemorrhages or choking of the disks. The arteries were not tortuous. The chest was normal. The heart rate was 84 per minute with a regular rhythm. The blood pressure was 170 systolic and 80 diastolic. The abdomen revealed no significant abnormalities. The upper extremities were flaccid with periods of spasticity. The

From the Institute of Pathology, Western Reserve University and University Hospitals, and the Department of Pathology, City Hospital.

1. Zimmerman, H. M., and German, W. J.: Arch. Neurol. & Psychiat. **30**: 309, 1933.
2. Stookey, Byron: Bull. Neurol. Inst. New York **3**:446, 1934.
3. Dandy, W. E.: Benign Tumors in the Third Ventricle of the Brain, Springfield, Ill., Charles C. Thomas, Publisher, 1933.
4. Masson, C. B.: Arch. Surg. **28**:527, 1934.
5. Jones, E. W., and Collins, D. H.: J. Neurol. & Psychopath. **15**:53, 1934.
6. Eskelund, V.: Ugesk. f. læger **97**:118, 1935.
7. Paterson, J. E., and Leslie, M.: Brit. M. J. **1**:920, 1935.

lower extremities were spastic with intermittent irregular twitchings. The patellar reflexes were exaggerated, with the left greater than the right. There was a positive Babinski sign bilaterally. The cranial nerves were normal as far as could be determined in an unconscious patient.

The urine contained sugar (3+) but no albumin. The spinal fluid was under markedly increased pressure, the stream projecting 10 cm. from the end of the needle. The spinal fluid was clear and colorless and contained 6 cells per cubic millimeter. Tests for globulin gave negative results. There was no increase in sugar. The Lange colloidal gold curve was normal, and the Wassermann reaction of the spinal fluid was negative.

At 8 p. m., one and one-half hours after admission, the patient's blood pressure had risen from 170 systolic and 80 diastolic to 204 systolic and 102 diastolic. One-half hour later the patient died.

The autopsy was done thirteen hours after death. Externally the brain revealed no abnormalities. On cut section the third ventricle was found to be

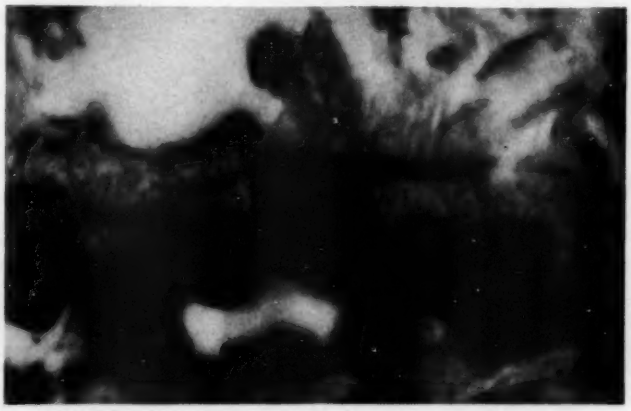

Fig. 1 (case 1).—Photomicrograph showing epithelial lining of cyst; × 1,800. Note the cilia projecting into the cyst contents and the line beneath the cilia formed by blepharoplasts.

completely filled by an ovoid cystic mass measuring 2 cm. in diameter, which dilated the ventricle causing it to impinge on the lateral ventricles. The cyst content consisted of a firm translucent jelly, near the center of which was a discrete ovoid opaque mass measuring 4 mm. in diameter. The cyst was suspended by a small stalk from the choroid plexus on one side of the roof of the third ventricle; it was possible to shell it completely out of the ventricle, leaving it suspended from the choroid plexus.

On section of the pons multiple small transverse areas of fresh hemorrhage were seen, which were bright red and exuded blood freely.

Microscopic sections through the cyst showed it to be lined with a multiple layer of cells similar to the ependymal layer, although a definite membrane could be traced to its choroid attachment. The nuclei were small, round or slightly oval and deeply staining, and the cytoplasm was eosinophilic and moderate in amount. Many of the lining cells were ciliated and contained a row of blepharoplasts. Several moderate-sized blood vessels entered the wall of the cyst by way of its

pedicle. The cyst content was structureless, acidophilic and hyaline, with scattered small nests of mononuclear cells. Near the center was an irregular, dense, finely granular mass.

Section of the pons showed multiple areas of recent hemorrhage about blood vessels. The walls of the blood vessels showed no abnormality, the exact points of rupture not being identified.

The pathologic diagnosis was: neuro-epithelial cyst of the third ventricle; recent pontile hemorrhages; cloudy swelling of the liver and kidneys; hyperemia of the spleen; arteriosclerosis, and healed pulmonary tuberculosis.

CASE 2.—A white man, 45 years of age, was admitted to the medical service of the City Hospital on Dec. 24, 1934, at 4:25 a. m. Five hours previously a severe headache had developed, which was accompanied by nausea. He complained of pain in the right side. He became short of breath, weak and passed into coma before admission.

He had had a chancre in 1911 and some antisyphilitic therapy in 1922. On Dec. 6, 1934, he had been hospitalized for two days with symptoms suggestive of chronic peptic ulcer. No history of symptoms referable to the head or central nervous system was elicited, and examination revealed only slight epigastric tenderness. The ocular fundi showed slight sclerosis.

Examination on the present admission revealed a well developed and well nourished middle-aged white man who was unconscious and breathed stertorously. The pupils were dilated and fixed to light. There was considerable papilledema. The chest and heart revealed no abnormalities. The blood pressure was 150 systolic and 86 diastolic and the pulse rate 84. The abdomen was normal. The patellar reflexes were hyperactive. No localizing neurologic sounds were found. The urine contained sugar (3+) and acetone (1+). The blood sugar was 228 mg. per hundred cubic centimeters and the carbon dioxide-combining power 48 volumes per cent. The blood urea nitrogen was 11.2 mg. per hundred cubic centimeters. Lumbar puncture revealed a spinal fluid pressure of 16 mm. of mercury. The spinal fluid cell count revealed 210 red cells per cubic millimeter and no white cells. A test for globulin gave negative results. The Wassermann reaction of the spinal fluid was negative, and the colloidal gold curve was normal. The Wassermann reaction of the blood was negative.

Insulin and stimulants were administered. The patient died seven hours after admission.

The autopsy was made five hours after death. The only significant observation in the thoracic and abdominal viscera was a chronic duodenal ulcer.

The dura mater was intact. The pituitary gland was normal in size, shape and consistency. The posterior clinoid processes were slightly flattened.

After fixation in dilute solution of formaldehyde U. S. P., the brain weighed 1,550 Gm. The left cerebral hemisphere appeared slightly larger than the right. The leptomeninges and vessels at the base of the brain showed no abnormalities. There was an extreme flattening of the entire cerebral cortex. At the base of the brain there was a bulging of the floor of the third ventricle, a rounded protrusion extending downward between the optic tracts, compressing these and the chiasm. The infundibulum was intact. On section there was found within the third ventricle a cyst measuring 3 by 2.5 by 2.5 cm. This was impacted in the posterior part of the chamber, obliterating the massa intermedia, separating and compressing the thalami, compressing the mamillary bodies and midbrain and obstructing the aqueduct of Sylvius. The foramina of Monro were not occluded but dilated, the right measuring 8 mm. in diameter and the left 7 mm. The anterior

part of the third ventricle was dilated. The lateral ventricles were markedly dilated. The cyst was free except for the line of attachment to the choroid plexus on each side of the roof of the third ventricle. The wall of the cyst was thin,

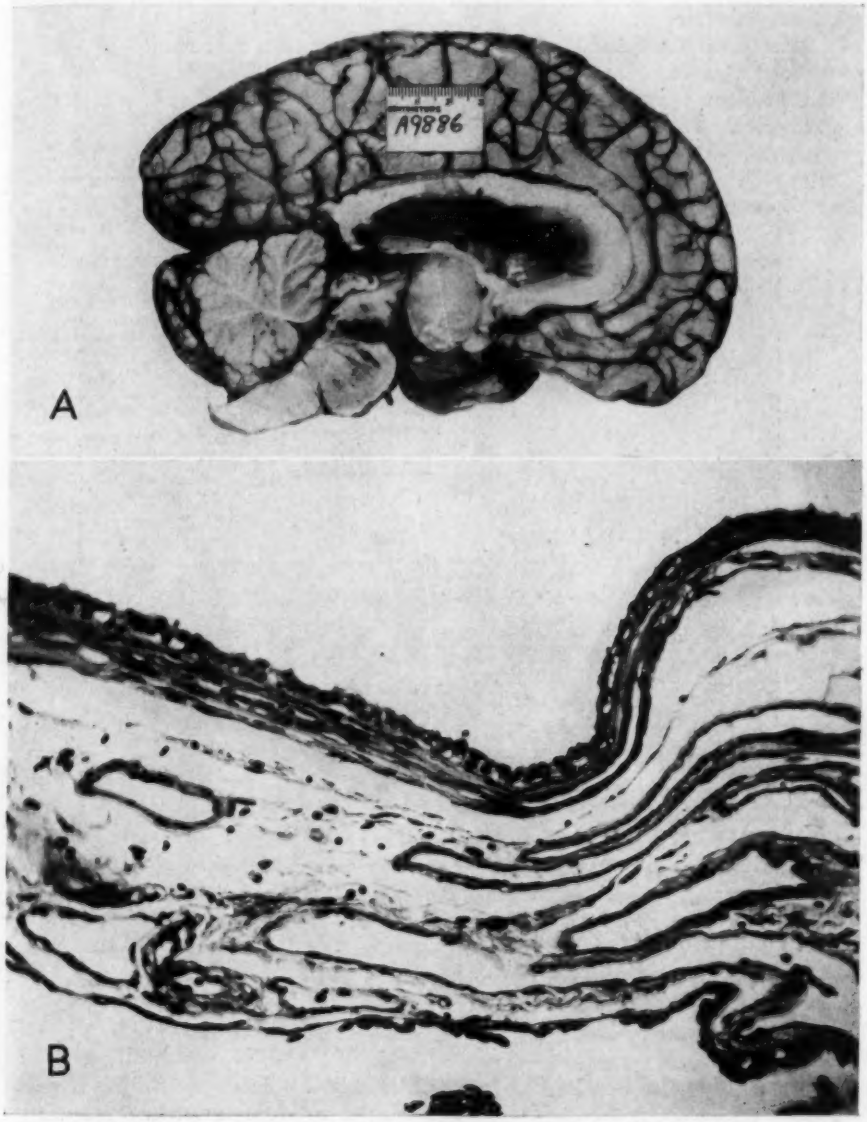

Fig. 2 (case 2).—*A*, median section of the brain showing the neuro-epithelial cyst within the dilated third ventricle; *B*, photomicrograph of the wall of the cyst; × 400.

transparent, well vascularized, and had smooth, glistening internal and external surfaces except that on the external surface fine granulations were present which

resembled tufts of choroid plexus. The cyst was filled with a firm, glistening, translucent, bluish-white, gelatinous material containing irregular opaque yellowish-white areas.

In the midbrain there was a fresh area of hemorrhage measuring 1.5 cm. in its greatest diameter.

Microscopically the wall of the cyst was made up of a thin, fibrous, well vascularized membrane lined by a continuous layer of flat and cuboidal cells. These cells were arranged in a single layer in some areas and in two or three layers in other areas. The nuclei were small, rounded and deeply staining. The cytoplasm was clear, eosinophilic and moderate in amount. Many of the cells were ciliated. Attempts to demonstrate blepharoplasts were unsuccessful. The outer layer of the wall merged insensibly with the choroid plexus. The material within the

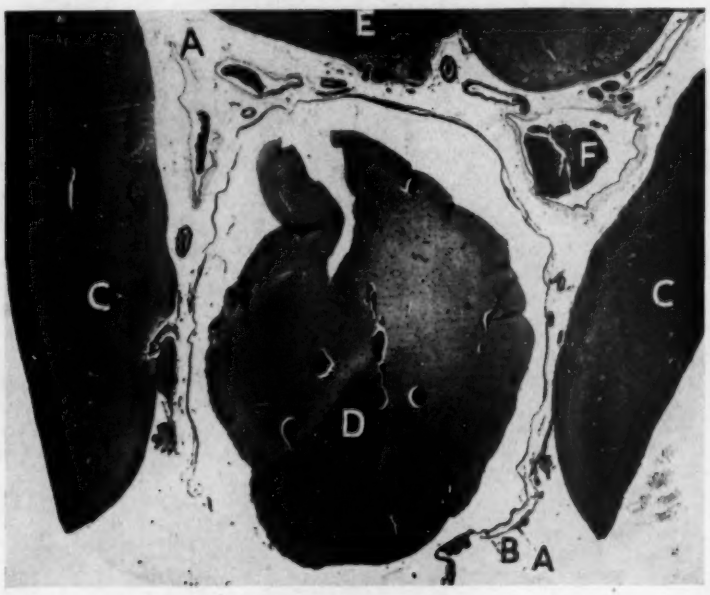

Fig. 3 (case 3).—Photomicrograph showing the cyst in situ with its surrounding structures: (*A*) tela choroidea, (*B*) wall of the cyst, (*C*) thalami, (*D*) contents of the cyst, (*E*) fornix, (*F*) thrombus in a small vein of Galen; × 10.

cyst was eosinophilic, smooth and homogeneous in some areas and finely granular in others. A moderate number of small mononuclear and polymorphonuclear cells were scattered throughout.

The pathologic diagnosis was: neuro-epithelial cyst of the third ventricle; obstructive internal hydrocephalus; recent hemorrhage in the midbrain; chronic passive hyperemia of the lungs, liver and kidneys, and chronic duodenal ulcer.

Case 3.—A white Czech of 49 years was admitted to Cleveland City Hospital on July 30, 1935 with a syndrome involving the posterior inferior cerebellar artery. He had become ill two days before admission. He died on Aug. 15, 1935. At autopsy there was a thrombosis of the left vertebral artery together with ischemic lateral softening of the medulla. An incidental observation was that of a spherical cystic structure 1 cm. in diameter occupying the anterior part of the third ven-

tricle. It was attached to the choroid plexus on the roof of the third ventricle immediately posterior to the foramina of Monro. The third ventricle was dilated only in its anterior portion and was not obstructed. The cyst did not obstruct the foramina of Monro, and the foramina and lateral ventricles were not dilated. The cyst was removed intact with its surrounding structures for histologic examination.

Multiple microscopic sections studied serially showed the cyst to lie entirely enclosed within the tela choroidea and to be separated from the third ventricle by the tissue of the tela choroidea and its ependymal covering. The outer wall of the cyst was nowhere in contact with the ependyma. The small veins of Galen were dilated and contained premortal thrombi. The wall of the cyst was thin, fibrous and lined by a single layer of ciliated cuboidal and columnar cells having small, round, deeply staining nuclei and a small amount of acidophilic cytoplasm. The material within the cyst was acidophilic, hyaline and homogeneous.

The pathologic diagnosis was: thrombosis of the left vertebral artery; ischemic lateral softening of the medulla; bronchopneumonia; generalized arteriosclerosis, and neuro-epithelial cyst of the third ventricle.

COMMENT

The neuro-epithelial cyst is a benign tumor which has its origin in a congenital defect of growth. The exact point of origin is at present disputed; the cyst may be derived from a rudimentary ependymal or choroidal structure. It is attached by a pedicle to the roof of the third ventricle and consists of a thin epithelized wall and a content of homogeneous acidophilic material, which is probably pseudomucin.

The incidence is higher in males (63 per cent) than in females and those in whom the lesion occurs are usually between 20 and 50 years of age. Only one of the reported cases occurred in a person under 18 years of age.

In case 1 it is possible that the headache which occurred six days before the fatal attack was due to a transient obstruction of the ventricular system by the tumor. If paroxysmal attacks had occurred over any considerable period of time, they obviously were considered unimportant by the patient. This is true also in case 2. In that case the ocular fundi appeared essentially normal three weeks before the sudden fatal obstruction. The pontile and midbrain hemorrhages were possibly caused by changes in pressure incidental to lumbar puncture.

Although none of the cases reported here permitted clinical diagnosis and treatment, analysis of the hitherto reported cases reveals that in 84 per cent symptoms were present for one month or more, that in 51 per cent symptoms were present for no longer than one year and that only in 14 per cent did death occur subsequent to the first observed attack. The characteristic signs and symptoms are produced by intermittent acute obstruction of the foramina of Monro or of the third ventricle by the cyst. This causes paroxysms of severe frontal headache, nausea, vomiting and papilledema. Relief or exaggeration of symptoms occurs on changes of posture. Diencephalic symptoms and signs have been present in numerous cases.

The treatment is surgical removal. Twelve operations with nine cures have been reported. The operation of choice is entrance into the lateral ventricle through the frontal lobe and removal of the tumor through the foramen of Monro.

SUMMARY

Three cases of neuro-epithelial cyst of the third ventricle are reported here, with the postmortem pathologic observations. Two of the patients died suddenly after exhibiting similar clinical manifestations, and neither had given a history of clinical manifestations previous to the fatal attack. In each there was found a characteristic cystic tumor forming an obstruction within the third ventricle. In the third patient the tumor was an incidental finding and had produced no obstruction.

The neuro-epithelial tumor is benign and originates in a congenital growth defect developing within the tela choroidea in the roof of the third ventricle. The associated signs and symptoms are chiefly those of intermittent acute obstruction in the ventricular system. The treatment is surgical removal.

General Review

SPONTANEOUS VIRUS DISEASES IN COMMON LABORATORY ANIMALS

(MICE, RATS, CATS AND MONKEYS)

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During the past few years, investigators have frequently reported the occurrence in certain common laboratory animals of spontaneous virus diseases and of analogous pathologic reactions. The majority of these conditions has been observed in the course of experimental work devised for other purposes. As is well known, the presence of an unrecognized virus disease in experimental animals has led to the erroneous interpretation of the results of certain investigations. For this reason it seemed desirable to review the growing literature dealing with spontaneous virus diseases and related conditions in mice, rats, cats and monkeys, in the hope that such a report might be helpful to those interested in biologic experimentation. In addition to the findings of others, pertinent observations which have been made over the past five years on these species have been included.

Some virus diseases, as they occur spontaneously in mature animals, are not associated with clinical manifestations; others have a distinctive syndrome. The latter type may exist in a supposedly healthy colony for generations. If the resistance of such a group becomes lowered (dietary deficiency, surgical intervention, etc.), the disease may become evident in an epizootic, with typical signs. Clinically, certain of the virus diseases simulate specific bacterial infections. A final diagnosis is based on the histopathologic observations, together with the experimental reproduction of the disease in a series of susceptible hosts by the exhibition of bacteriologically sterile emulsions or filtrates of specific tissues from an affected animal, or on the demonstration in recovered animals of immunity to, or immune substances for, the suspected virus.

Pathologic reactions simulating the response effected by different known viruses have been observed from time to time. The analogy in the greater number was based on the presence in the lesions of

This work was aided by a grant from the Rockefeller Foundation.

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inclusion-bearing cells. Recently, protoplasmic bodies, practically indistinguishable from virus inclusions, have been produced in vivo [1] and in vitro [2] by different physicochemical agents; similar intracellular masses have been observed in the cells of the seminal vesicles [3] and in the nucleus supra-opticus and the nucleus paraventricularis [4] of normal animals and of man. Although these observations cast doubt on the view that all intracellular inclusions are due to the activity of a virus, in biologic experimentation it is possibly the wiser course to make an effort to ascertain the nature of the causative agent when one incidentally notes lesions which seem to have been caused by a virus.

Attention is directed to the different spontaneous virus diseases and analogous conditions encountered in mice, rats, cats, and monkeys.

GROUP I

The first group is comprised of diseases with cell inclusions which are transmissible and in which the filtrability of the causative agent is established.

MICE.—*Infectious Ectromelia.*[5]—The condition was first described by Marchal in 1930 and later by McGaughey and Whitehead in Great Britain. Two forms of the spontaneous infection have been noted: 1. The cutaneous or generalized type is characterized by the development of localized cutaneous lesions on the foot or tail, ultimately progressing to gangrene, with loss of the affected part, and recovery or to a more widespread cutaneous involvement and death. Clinically, the picture resembles ergot poisoning or the specific bacterial infections reported by Levaditi and his co-workers,[6] Mackie, Van Rooyen and Gilroy [7] and Strangeways.[8] 2. In mice with the abdominal type of the disease,

1. Lee, J.: Proc. Soc. Exper. Biol. & Med. **31**:383, 1933.

2. (*a*) Akiyma, S.: Arb. a. d. Med. z. Okayama **1**:278, 1929. (*b*) Davenport, H. A.; Ranson, S. W., and Terwilliger, F. H.: Anat. Rec. **48**:251, 1931. (*c*) Heinbecker, P., and O'Leary, J. L.: ibid. **45**:219, 1930.

3. Heidenhain, M., and Werner, F.: Ztschr. f. Anat. u. Entwcklngsgesch. **72**: 556, 1924. Ludford, R. J.: Proc. Roy. Soc., London, s.B **98**:354, 1925.

4. Scharrer, E., and Gaupp, R.: Ztschr. f. d. ges. Neurol. u. Psychiat. **148**: 766, 1933.

5. (*a*) Barnard, J. E., and Elford, W. J.: Proc. Roy Soc. London, s.B. **109**: 306, 1931. (*b*) Downie, A. W., and McGaughey, C. A.: J. Path. & Bact. **40**:147 and 297, 1935. (*c*) Marchal, J.: ibid **33**:713, 1930. (*d*) McGaughey, C. A., and Whitehead, R.: ibid. **37**:253, 1933.

6. Levaditi, C.; Nicolau, S., and Poincloux, P.: Compt. rend. Acad. d. sc. **180**:1188, 1925. Levaditi, C., and Selbie, F. R.: ibid. **189**:1332, 1929. Levaditi, C.; Selbie, F. R., and Schoen, R.: Ann Inst. Pasteur **48**:308, 1932.

7. Mackie, T. J.; Van Rooyen, C. E., and Gilroy, E.: Brit. J. Exper. Path. **14**:132, 1933.

8. Strangeways, W. I. : J. Path. & Bact. **37**:45, 1933.

cutaneous lesions are absent at death. The most outstanding observations at necropsy are: increased intraperitoneal and pleural fluid; engorgement of the gastro-intestinal tract, and enlargement, together with numerous areas of grayish-white mottling, of the liver and the spleen. Histopathologically, characteristic cytoplasmic inclusions have been observed in the epithelium and subcutaneous tissues subjacent to the superficial lesions, the wall of the intestine, the zymogenous cells of the pancreas, the mucoid acini of the salivary glands, the liver, the spleen and the lymph nodes. Many of the involved areas show an associated chronic inflammatory reaction.

The filtrability of the causative agent has been demonstrated, and the diameter of the virus particles has been estimated to be from 0.1 to 0.15 micron. Cultivation of the virus in vitro has been carried out. The host range has not been reported.

Hepatopathy of Mice of the Clacton Strain.[9]—In 1932 Findlay reported the condition in apparently normal mice of the Clacton strain. The disease was manifest in the presence of intranuclear inclusions in many of the hepatic cells. A similar histologic reaction was produced in other strains of mice by bacteria-free filtrates of the livers of mice of the Clacton strain. The host range has not been ascertained.

MONKEYS.—*Virus B and Herpes.*—The clinical signs and the pathologic changes which may occur in the course of the spontaneous disease due to infection with either virus have not been described. However, it is believed infection with either virus is indigenous to the species. Experimental evidence in substantiation of this view is: (1) the isolation of virus B from a human patient who died following a bite by a monkey;[10] (2) the difficulty of inducing an infection with virus B or herpes in certain Macacus rheus monkeys,[11] and (3) the demonstration of antiviral antibodies for these viruses in the serum of some apparently normal monkeys.[12]

Virus B:[13] The clinical picture presented by monkeys artificially infected varies with the site of inoculation. When the intracutaneous route is used, a localized hemorrhagic or vesiculopustular cutaneous lesion develops. Intravenous injection produces an exanthem, most marked on the forehead, eyes and face, and an associated exanthem chiefly evident on the buccal mucous membrane, tongue, palate and

9. Findlay, G. M.: Brit. J. Exper. Path. **13**:223, 1932.

10. Sabin, A. B., and Wright, A. M.: J. Exper. Med. **59**:115, 1934.

11. (*a*) Gay, F. P., and Holden, M.: J. Infect. Dis. **53**:287, 1933. (*b*) McKinley, E. B., and Douglas, M.: ibid. **47**:511, 1930. (*c*) Sabin, A. B.: Brit. J. Exper. Path. **15**:321, 1934. (*d*) Zinsser, H.: J. Exper. Med. **49**:661, 1929.

12. Sabin.[11c] Gay and Holden.[11a]

13. Sabin, A. B.: Brit. J. Exper. Path. **15**:268, 1934. Sabin, A. B., and Hurst, E. W.: ibid. **16**:133, 1935. Sabin.[11c] Sabin and Wright.[10]

conjunctiva. Monkeys infected intracerebrally show a variable clinical picture, as is usual with other forms of myelo-encephalitis. Nystagmus, somnolence and epileptiform convulsions may be evident, with concomitant progressive paresis and spastic paralysis. Histopathologically, acidophilic intranuclear inclusions are seen in the various lesions and are apparent in cells of ectodermal, endodermal and mesodermal origin.

Virus B is filtrable. Results of cross-neutralization tests have indicated that there is an immunologic relationship with the virus of herpes and with that of pseudorabies. The host range includes rabbits, guinea-pigs, mice and man.

Herpes:[14] Monkeys into which herpes virus has been injected intracerebrally may show either acute manifestations or a much more chronic condition resembling epidemic encephalitis in man. In monkeys with acute manifestations, pathologic studies of the central nervous system reveal: widespread cortical involvement, with many of the ganglion cells presenting acidophilic intranuclear inclusions, degenerative changes and necrosis, and infiltration of the perivascular spaces and meninges by acute inflammatory cells. Histopathologically, the animals with the chronic form present: mononuclear cuffing of the vessels of the cerebral cortex, basal ganglions, pons, medulla and leptomeninges; intranuclear inclusions in a small number of mononuclear and ganglion cells; degenerative changes involving the ganglion cells, and gliosis.

The herpes virus is filtrable. The rabbit, guinea-pig and mouse and man are susceptible to infection.

GROUP II

The second group is comprised of diseases with cell inclusions which are transmissible but in which the filtrability of the causative agent has not been definitely established.

MICE AND RATS.—*Disease of the Salivary Glands.*[15]—The conditions are evidenced in apparently healthy mice and rats by the presence in the salivary glands of chronic inflammatory cell foci, together with intranuclear and cytoplasmic inclusions in many of the cells of the ducts and parenchymatous tissue. The submaxillary glands usually show the most marked involvement. The disease in both rats and mice has been successfully transmitted to susceptible hosts in series by bacteriologically sterile emulsions of involved salivary glands. The infection is species-

14. Rivers, T. M.: Filterable Viruses, Baltimore, Williams & Wilkins Company, 1928. Zinsser.[11d]

15. (*a*) Kuttner, A. G., and T'ung, T.: J. Exper. Med. **62**:805, 1935. (*b*) Kuttner, A. G., and Wang: ibid. **60**:773, 1934. (*c*) Thompson, J.: J. Infect. Dis. **50**:162, 1932; (*d*) Am. J. Path. **10**:676, 1934; (*e*) J. Infect. Dis. **58**:59, 1936.

specific. In infected rats, histologically normal kidneys, lymph nodes and lungs apparently harbor the virus.

GROUP III

The third group is comprised of diseases without cell inclusions which are transmissible and in which the filtrability of the causative agent is established.

MICE AND MONKEYS.—*Lymphocytic Choriomeningitis.*[16]—That the disease is harbored by apparently normal mice and monkeys is evident by the fact that the condition was encountered as an incidental complication in the investigations of Armstrong and Lillie and of Traube. For this reason, it is believed that the disease occurs spontaneously in these species.

Clinically, in monkeys infected intracerebrally there develop on from the eighth to the fifteenth day slight fever, dejection and loss of appetite and of weight. As the disease progresses, signs of meningeal involvement are apparent in stiffness and in transient tetanus-like convulsions. The spinal fluid is under increased pressure; cell counts and smears reveal the presence of lymphocytosis. The majority of animals recover. Histopathologically, the characteristic observations are: a more or less marked cellular invasion of the meninges and the choroid plexuses, with lymphocytes predominant; perivascular infiltration, usually restricted to the perforating and subependymal vessels and slight parenchymatous degeneration and hyperplasia of the reticulo-endothelial cells of the liver, spleen and lymph nodes.

Artificially infected mice show clinical signs on from about the fifth to the tenth day, with somnolence, photophobia, tremor and spasm of the limbs. Histopathologic study reveals in the meninges and in the choroid plexuses changes similar to those noted in monkeys, but the reaction is usually much more marked, and, in addition, there is necrosis of some of the nerve cells of the cerebrum, cerebellum, brain stem and spinal cord.

The disease has been passed serially in the respective species, and results of cross-neutralization tests have indicated that the causative agents are serologically identical. Filtrability has been demonstrated. Guinea-pigs and man are susceptible to infection.

CATS.—*Distemper.*—Epizootics occur among uncaged animals in certain years during the late fall, winter and early spring. If such conditions prevail, the disease breaks out frequently in the laboratory when

16. Armstrong, C., and Dickens, P. F.: Pub. Health Rep. **50**:831, 1935. Armstrong, C., and Lillie, R. D.: ibid. **49**:1019, 1934. Rivers, T. M.: Am. J. Path. **11**:826, 1935. Rivers, T. M., and Scott, T. F. N.: Science **81**:439, 1935. Traube, E.: ibid. **81**:298, 1935; Am. J. Path. **11**:825, 1935.

apparently normal cats are confined in relatively cramped quarters. The majority of the cats will show signs of infection; few are clinically resistant. Of the animals affected, the greater number decline rapidly and die within a few days; some survive over a longer period; the remainder usually live for months, with an apparently chronic disorder (these probably serve as a reservoir of infection in nature). Clinically, the acute spontaneous infection falls essentially into two types: (1) moist (pneumotropic) and (2) dry (neurotropic) distemper. In the present report, the remarks will be confined to observations on the dry type of distemper.

The early clinical signs in a typical instance of dry distemper are those of severe conjunctivitis complicated occasionally by a mild infection of the upper respiratory tract. As the disease progresses, the animal shows: high fever, diaphragmatic spasms, regurgitation of food, rapid loss of weight, development of an ataxic gait and, when walking, a tendency to fall from side to side. Occasionally strabismus and other ocular signs are present. Eventually, the cat refuses to eat, drink or move. Some animals prior to death show opisthotonos and have convulsions.

At autopsy, the typical observations in animals killed by ether when almost moribund are: purulent conjunctivitis, congestion of the meninges, a moderate increase in the cerebrospinal fluid and congestion of the brain stem and the cervical and lumbar regions of the spinal cord. On gross examination of the section, minute hemorrhages are noted almost invariably in the superior and inferior colliculi; petechial hemorrhages are also irregularly scattered throughout the brain stem and the gray matter of the cervical and lumbar regions of the cord. Cell counts and smears made of the spinal fluid obtained by cisternal puncture at necropsy in the majority of cases show lymphocytosis. Aerobic and anaerobic cultures of heart blood, spinal fluid and tissues of the brain and the cord rarely show bacterial growth.

Microscopic study of sections from the brain and spinal cord of typical specimens discloses comparable observations except for a slight variation from animal to animal (apparently dependent on the virulence of the infection and the duration of the disease). The most marked reaction is usually seen in sections from the basal ganglions, midbrain and medulla. The lesions vary from section to section and from field to field. The characteristic observations are: multiple diapedetic and petechial hemorrhages; associated edema of the subjacent tissues; irregulary scattered foci of mononuclear cells; cuffing of some of the vessels by similar cells and degenerative changes and necrosis, together with neuronophagia, of a small number of nerve cells. Sections from different parts of the cerebral hemispheres and cerebellum usually show

little reaction; occasionally, congestion of the vessels and hemorrhages of various sizes are present in certain areas. The gray matter of the cervical and lumbar regions of the cord shows congestion and diapedetic hemorrhages; in these regions of the cord external and internal pachymeningitis, characterized by an infiltration of mononuclear cells, may be associated.

The dry type of distemper has been reproduced experimentally in apparently normal cats in series by the intracerebral injection of bacteria-free emulsions or Seitz filtrates of tissue of the brains and cords of affected cats.

GROUP IV

The fourth group is comprised of diseases without cell inclusions which are transmissible but in which the filtrability of the causative agent has not been definitely established.

MICE.—*Theiler's Disease.*[17]—This condition occurs spontaneously and is characterized by flaccid paralysis of the hind legs. Knowledge of the disease is based on the artificially induced infection produced in normal mice by the intracerebral injection or nasal instillation of bacteria-free suspensions of tissue of the brains of affected mice. The animals in most instances have an ascending type of paralysis; young mice may die without showing clinical signs. The histopathologic observations are: acute necrosis of the ganglion cells of the anterior horns of the spinal cord and of isolated ganglion cells of the cerebrum, marked neuronophagia and perivascular infiltration of the cerebrum and spinal cord. Monkeys are resistant to the disease. Further investigation of the host range has not been reported.

Lymphatic Leukemia.[18]—As a rule, lymphatic leukemia of mice has not been grouped with the diseases caused by a virus. However, in view of the existing evidence, it is believed that this condition should be considered in group IV.

The literature is mute evidence that spontaneous leukemia causes as varied a picture in mice as in man. Since most of the experimental studies have been carried out on animals with the lymphatic type of leukemia, the remarks will be confined to this form of the condition in mice. The spontaneous disease causes readily definable changes;

17. Theiler, M.: Science **80**:122, 1934.

18. Ellermann, V.: The Leucosis of Fowls and Leucemic Problems, London, Gyldendal, 1922. Ellermann, V., and Bang, O.: Ztschr. f. Hyg. u. Infectionskr. **63**:231, 1909. Haaland, M.: Ann. Inst. Pasteur **19**:165, 1905. MacDowell, E. C., and Richter, M. N.: Proc. Soc. Exper. Biol. & Med. **28**:1012, 1931. Opie, E. L.: Medicine **7**:31, 1928. Parsons, D.: J. Path. & Bact. **40**:45, 1935. Potter, J. S., and Richter, M. N.: Arch. Path. **15**:198, 1933. Richter, M. N., and MacDowell, E. C.: Proc. Soc. Exper. Biol. & Med. **26**:362, 1929; J. Exper. Med. **51**:659 and 823, 1930. Simmonds, J. P.: J. Cancer Research **9**:329, 1925.

marked lymphocytosis in the blood, with an increase in the number of larger than normal lymphocytes apparent in smears, and enlargement of the spleen and many of the lymph nodes. Histologically, the spleen and lymph nodes show obliteration of the normal architecture, owing to infiltration by white blood cells; the liver, lungs and kidneys usually show evidence of leukemic infiltration, which is chiefly perivascular.

Although leukemia in fowls has been reproduced by cell-free filtrates, the findings of the majority of investigators indicate that at least two factors are prerequisite to inducing the condition artificially in mice: (1) a hereditary predisposition and (2) the introduction into a susceptible host of a certain number of leukemic cells in the bacteria-free suspensions of the specific tissues from an affected mouse (suspensions of tissue which are commonly employed in inducing virus diseases artificially contain a certain number of intact cells unless they are adequately centrifugated). From histologic studies of animals with induced leukemia, Potter and Richter concluded that the condition is effected by proliferation of the leukemic cells which have been introduced. In contrast, Parsons described a leukemic condition which was transmitted through twenty passages by grafts of a spindle cell sarcoma originally produced by di-benzanthracene. The histologic response in affected mice was suggestive of proliferation of fixed tissue cells rather than of those introduced with the graft.

GROUP V

The fifth group is composed of miscellaneous conditions in which the specific etiologic factor has not been demonstrated but in which the changes have been considered analogous to the response effected by some of the known viruses.

MICE.—A. Thompson in America [15d] reported the occurrence of an epizootic in mice resembling clinically and in observations at necropsy the cutaneous or the generalized type of infectious ectromelia. The condition differed from the latter disease in the absence of inclusions in the epithelial tissues and in the presence of intranuclear and cytoplasmic inclusions in many of the parenchymatous cells of the liver, in association with a focal chronic inflammatory reaction.

B. Intranuclear changes comparable to those of hepatopathy of mice of the Clacton strain have been noted in a small number of apparently normal mice.[15d]

RATS.—A. Hindle [19] and Hindle and Stevenson [20] described the presence of intranuclear inclusions in the cells of the renal tubules of apparently healthy wild London rats and of rats from other localities

19. Hindle, E.: Nature **129**:796, 1932.

20. Hindle, E., and Stevenson, A. C.: Tr. Roy. Soc. Trop. Med. & Hyg. **23**: 327, 1930.

possibly in contact with sewage and refuse. Comparable changes were produced in normal rats by contact with sewer rats and by the exhibition of filtrates of sewage in 10 per cent nutrient broth.

B. Intranuclear bodies in the absence of an inflammatory reaction have been reported as involving the cells of the facial portion of the parotid gland of rats.[15d] The inclusions were considered comparable to those in the livers of mice of the Clacton strain, mentioned previously, and different from those of the salivary gland (these did not occur concomitantly).

CATS.—A. In the cerebral tissue of a small number of apparently normal cats cuffing of many of the vessels with chronic inflammatory cells has been noted. The reaction was considered to resemble closely the changes in human patients with epidemic encephalitis. Reactions were not effected in other normal cats by the intracerebral injection of bacteria-free emulsions or Seitz filtrates of the brain tissue of affected animals.

B. Observations on leukemia in cats have been reported.[21]

MONKEYS.—A. Stewart and Rhoads [22] noted the presence of intranuclear inclusions in the inflamed nasal mucosa in a considerable number of monkeys with acute poliomyelitis. Similar intranuclear masses, associated frequently with infiltration of inflammatory cells, were observed by Covell [23] in normal and in diseased monkeys, involving the nasal mucosa, the trachea, the bronchioles, the alveoli of the lungs and the bile ducts.

B. Perivascular reactions simulating those observed in tissue of subjects with epidemic encephalitis have been observed in the cerebral tissue of a small number of normal monkeys.[23a]

C. Massaglia [24] reported a case of spontaneous leukemia in a monkey.

D. Inclusion bodies similar to those noted in guinea-pigs [25] and in certain rodents [26] have been observed in the parotid and submaxillary glands of Cebus fatuelles monkeys.[27]

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21. Siedamgrotsky: Ber. u. d. Veterinärw. im Königr. Sachs. **16**:64, 1871; quoted by Opie.[18]

22. Stewart, F. W., and Rhoads, C. P.: Proc. Soc. Exper. Biol. & Med. **26**: 664, 1929.

23. Covell, W. P.: Am. J. Path. **8**:151, 1932.

23a. Lucke, B.: Arch. Neurol. & Psychiat. **10**:212, 1923.

24. Massaglia, A. C.: Lancet **1**:1056, 1923.

25. Cole, R., and Kuttner, A. G.: J. Exper. Med. **44**:855, 1926.

26. Rector, L. E., and Rector, E. J.: Am. J. Path. **10**:629, 1934. Kuttner and Wang.[15b] Thompson.[15c, d]

27. Cowdry, E. V., and Scott, G. H.: (*a*) Proc. Soc. Exper. Biol. & Med. **32**:709, 1935; (*b*) Am. J. Path. **11**:659, 1935.

E. In Macacus rhesus monkeys, inclusion-bearing cells have been described in the parenchyma of the kidneys by a few investigators.[28]

SUMMARY

The various diseases caused by viruses and analogous conditions in mice, rats, cats and monkeys have been briefly summarized. In the past, experimental studies have been devoted almost exclusively to the investigation of the more pathogenic viruses. However, those which do not show distinctive clinical signs are of interest. That such viruses may be potentially pathogenic should not be overlooked. The recent demonstration of an etiologic factor in two cases of aseptic meningitis in man identical with the virus-producing lymphocytic choriomeningitis in mice and monkeys, as well as the isolation of virus B from a person in whom an ascending paralysis developed and who died following a bite by a monkey, suggests that the animal kingdom is an incompletely probed reservoir of human virus disease.

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28. Cowdry, E. V.; Lucas, A. M., and Fox, H.: Am. J. Path. **11**:237, 1935. Cowdry and Scott.[27b] Hindle.[19]

Notes and News

The Elizabeth Clay Howald Scholarship.—This scholarship was endowed by the late Ferdinand Howald, an alumnus of Ohio State University, in memory of his mother, Elizabeth Clay Howald. Appointments will be made annually, and the scholar will receive an honorarium of $3,000, paid in twelve equal monthly instalments.

Any person who has shown marked ability in some field of study and has in progress work the results of which promise to be an important contribution to pathology shall be deemed eligible to appointment to this scholarship.

If the scholar has ever been a student of Ohio State University or a member of the university staff, he may carry on his investigation either at Ohio State University or, subject to the approval of the Graduate Council, elsewhere, either in this country or abroad where superior advantages for his particular field of study are available. If the scholar has never had any connections with Ohio State University, however, he must carry on his investigation at this university.

Prospective candidates may secure application blanks by addressing the Dean of the Graduate School, Ohio State University. Applications must be filed with the Dean of the Graduate School not later than March 1. The appointment will be made on April 1, and the term of appointment will begin June 1.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

The Experimental Production of a Collateral Circulation to the Heart. C. S. Beck and V. L. Tichy, Am. Heart. J. **10**:849, 1935.

The available extracardiac bed for vascularization of the myocardium consists of the pericardium, pericardial fat, mediastinal tissues, pleura, diaphragm, substernal muscles, the musculature of the thoracic wall and the omentum. In these experiments the pericardium, the pericardial fat and the mediastinal tissues were utilized for the vascular bed. Direct continuity between the vascular bed and the heart was established by the production of adhesions between the pericardium and the heart. The stimulus necessary to bring about vascular continuity between the extracardiac and cardiac beds was a reduced or subnormal pressure in the coronary bed. The pressure was reduced by occluding the coronary arteries by clips of sheet silver. This occlusion can be produced either slowly by successive operations or by complete ligation in one stage. The anastomoses between the extracardiac vascular bed and the heart were determined by injecting ferric ferrocyanide. The dye was injected into the collateral bed from the aorta—the ostia of the coronary arteries and the thebesian channels being excluded from the injection system. The coronary arteries and the myocardium were then examined grossly and microscopically for the presence of the dye. Almost total occlusion of both coronary arteries was effected with recovery when a collateral vascular bed was present. Partial occlusion of the coronary arteries was better tolerated, however, if the heart had been given a collateral bed at a previous operation. On the basis of this evidence the presence of a collateral circulatory bed can be considered as a prophylaxis against the ravages of sudden occlusion of the major coronary arteries. These experiments were made on dogs. One human patient has been the subject of similar procedures.

R. J. McNamara.

The Production of a Collateral Circulation to the Heart: Pathological Anatomical Study. R. Moritz and C. S. Beck, Am. Heart J. **10**:874, 1935.

On the assumption that thrombotic occlusion of a major coronary artery can be recognized clinically, it is probable that the diagnosis could have been made during life in eight of the ninety-four patients cited in this article. Only fourteen of the patients died as the direct result of the first major coronary occlusion. Concerning the eighty patients who survived the first occlusion, the question is whether or not they could have been protected against disastrous results from subsequent occlusions by the production of an extracardiac coronary collateral circulation. In thirty-seven of these eighty patients, concomitant disease or continued cardiac incompetence rendered the operative risk unjustifiable. The remaining forty-three patients might have been benefited by the production of the extracardiac collateral circulation. With the exception of seven patients who died of other causes, all died of subsequent attacks of coronary thrombosis. The data, chiefly pathologic, derived from this study of ninety-four cases of major coronary arterial occlusion indicate that there was a period after the first coronary occlusion in the lives of forty-three patients when the production of extracardiac coronary collateral circulation might have been feasible and beneficial.

From the Authors' Summary.

STRUCTURAL CHANGES IN THE PITUITARY OF THE THYROIDECTOMIZED RAT. I. T. ZECKWER, I. W. DAVISON, T. B. KELLER and C. S. LIVINGOOD JR., Am. J. M. Sc. **190**:145, 1935.

When thyroidectomy is performed in a young rat there results stunting of body growth; an increased weight of the pituitary due both to increased solids and increased fluid content; a marked reduction or nearly complete disappearance of acidophils; an increase in the number of basic-staining cells, and the appearance of great numbers of large cells filled with hyaline substance. These "thyroidectomy cells" appear, according to the special staining technic used, to be transformed cells containing blue granules. The "thyroidectomy cells" appear to be secreting and storing a secretory product which is hyaline in appearance. It is suggested that the stunting of body growth in the cretin rat may be due to loss of acidophils of the pituitary, which in turn depends on loss of the thyroid secretion. Acidophils seem to disappear by degranulation rather than by frank degeneration. An abundance of thyrotropic hormone was found to be present in certain pituitaries depleted of acidophils, a finding which rules out the acidophil cell as the producer of thyrotropic hormone. Since there is no atrophy of the adrenals in certain rats, it is reasonable to consider that the acidophils cannot be the producers of the adrenotropic hormone. When thyroidectomy is incomplete, the changes described are slight or absent.

FROM THE AUTHORS' SUMMARY.

NUCLEAR INCLUSIONS. E. V. COWDRY and G. H. SCOTT, Am. J. Path. **11**:647 and 659, 1935.

Intranuclear inclusions, closely resembling those caused by salivary gland viruses, were found in the parotid and submaxillary glands of 2 monkeys (Cebus fatuellus) which had received viosterol but which showed no signs of disease. No inclusions were observed in the salivary glands of 18 monkeys (Macacus rhesus) similarly treated.

Intranuclear inclusions suggestive of virus action were found in the kidneys of 12 of 16 monkeys (Macacus rhesus) given repeated doses of viosterol, of 1 of 10 normal controls, and of 18 of 107 pathologic controls consisting of animals employed in the laboratory in a variety of experiments but not given viosterol.

FROM THE AUTHORS' SUMMARIES.

NEUROPATHOLOGY OF EXPERIMENTAL VITAMIN DEFICIENCY. M. C. L. GILDEA and OTHERS, Am. J. Path. **11**:669, 1935.

Seventeen dogs given a diet deficient in vitamin B (Cowgill) showed signs of acute disturbance of the central nervous system and died without treatment with vitamin B concentrates. Only minimal histologic changes were found in the central nervous system. Eight dogs which were given a similar diet but in which the acute neurologic signs were repeatedly and temporarily relieved with vitamin B concentrates began gradually to show a residual degree of spastic ataxia and eventually motor paralysis, with reflexes present. Definitive histologic lesions of the central nervous system were found in all but one animal. Nissl stains of the cerebral and Purkinje cells and of the ventral horn cells revealed evidence of degeneration. Weigert-Pal stains of the spinal cord showed definite losses of myelin in seven dogs. The peripheral nerves of three dogs showed an increase of material staining with scarlet red or by the Marchi technic. Observations on the effects of partial starvation, of supplements of cod liver oil and of therapy with dried yeast on the morphologic changes in the central nervous system were rendered inconclusive because, probably, the basic deficiency was not sufficiently prolonged to produce morphologic changes in the nervous system of any of the animals in such experiments.

FROM THE AUTHORS' CONCLUSIONS.

EXPERIMENTAL CONCENTRIC AND ECCENTRIC CARDIAC HYPERTROPHY IN RATS. D. A. RYTAND and W. DOCK, Arch. Int. Med. **56**:511, 1935.

With a modification of Chanutin's technic for bringing about renal insufficiency, cardiac hypertrophy was produced in a group of albino rats; the hearts were compared with normal hearts and with hearts in which hypertrophy had been induced by the feeding of thyroid. The results confirmed and extended those of Chanutin. The heart of the rat reacts to the arterial hypertension of renal insufficiency by concentric hypertrophy of the left ventricle. In concentric hypertrophy so induced, the myocardial fibers of the left ventricle are increased in width and decreased in length. The heart of the rat reacts to artificial hyperthyroidism by eccentric hypertrophy involving the two ventricles equally. In eccentric hypertrophy so induced, the myocardial fibers do not show a significant change in width but lengthen greatly. A consideration of cardiodynamics shows that concentric hypertrophy is the optimum compensatory mechanism for the heart of the subject with hypertension and eccentric hypertrophy for the heart of the subject with hyperthyroidism.

FROM THE AUTHORS' SUMMARY.

FUNCTIONAL CHANGES IN THE BRAIN OF THE DOG AFTER REDUCTION OF THE CEREBRAL BLOOD SUPPLY. L. A. ANDREYEV, Arch. Neurol. & Psychiat. **34**:699, 1935.

By means of conditioned reflexes Andreyev studied the effect of severe cerebral anemia on the mental activity of dogs. Anemia was produced by ligation of certain arteries (the carotid, vertebral, subclavian and other arteries) as described by him in a previous contribution (*Arch. Neurol. & Psychiat.* **34**:481, 1935). As indicator of the conditioned reflexes, either the motor or the salivary reaction was used. In two dogs the ligation of the arteries was performed before the beginning of the experiments with the conditioned reflexes; in two others, the vessels were tied after the normal activity of the brain had been studied by means of the conditioned reflexes. Depending on the numbers of arteries ligated, that is, on the extent of the anemia produced, the disturbances created in the higher mental activity of the animals varied. They were temporary or permanent, strong or weak, and in some instances the function even returned to normal. Another conclusion arrived at was that both fundamental nervous processes, excitation and inhibition, are weakened after ligation of the arteries supplying the brain.

GEORGE B. HASSIN.

THE BLOOD CHOLESTEROL OF RABBITS IN RELATION TO ATHEROSCLEROSIS. K. B. TURNER and E. H. BIDWELL, J. Exper. Med. **62**:721, 1935.

The action of potassium iodide in preventing a significant rise in the blood cholesterol of rabbits fed cholesterol was temporary. After about four months it lost its effectiveness, and the blood cholesterol rose. In rabbits with hypercholesteremia resulting from long continued cholesterol feeding, the administration of potassium iodide caused a marked rise in the blood cholesterol. On the other hand, dried whole thyroid given to such animals produced a sharp fall in the blood cholesterol. This fall was temporary and was followed by a rise to new high levels. In thyroidectomized rabbits fed cholesterol and potassium iodide, both thyroid and thyroxin delayed but did not prevent a rise in the blood cholesterol. Even with the hypercholesteremia in these animals, however, the incidence of atherosclerosis was low. Age apparently played a part in determining the response of the blood cholesterol to cholesterol feeding. In a group of old rabbits when compared with a younger group the rise in the cholesterol of the blood was greater and the subsequent return toward normal was slower when the feeding was stopped.

FROM THE AUTHORS' SUMMARY.

Pathologic Anatomy

GALLSTONE FORMATION ABOUT EGGS OF CLONORCHIS SINENSIS. W. FISCHER, Centralbl. f. allg. Path. u. path. Anat. **63**:164, 1935.

In the body of a Chinese woman, 55 years old, fifty worms (Clonorchis sinensis) plugged the common bile duct and occurred in the thickened dilated intrahepatic ducts to the periphery of the liver. In the gallbladder a soft stone 11 mm. in diameter was found which consisted of bile pigment and calcium with only traces of cholesterol. About a third of the center of the stone was composed of eggs of Clonorchis. The composition of the stone bespoke an origin in the bile ducts of the liver, an origin which was in the nature of a simple incrustation of a foreign body. The importance of epithelial hyperplasia of the bile ducts in the development of carcinoma is mentioned.

GEORGE RUKSTINAT.

NECROSIS OF THE LUNG DUE TO COMPRESSION OF THE PULMONARY ARTERY. K. HASSLER, Frankfurt. Ztschr. f. Path. **46**:385, 1934.

A 48 year old man had a condition diagnosed as recurrent endocarditis of the mitral and aortic valves and pulmonary infarction. The autopsy revealed a dissecting aneurysm of the ascending aorta which had compressed the right pulmonary artery and resulted in necrosis (infarction) of the right lung. The bronchial arteries could not supply the lung sufficiently to prevent the occurrence of the infarct.

OTTO SAPHIR.

HONEYCOMBED LUNGS IN SYPHILIS. K. TAUBER, Frankfurt. Ztschr. f. Path. **46**:431, 1934.

A 51 year old man whose blood showed a four plus Wassermann reaction and who had typical syphilitic ulcers of the scrotum and of both forearms died of glomerulonephritis which followed erysipelas of the face. Severe changes in the right lung were found on roentgen examination. The autopsy revealed lobar pneumonia in the middle and upper lobes of the right lung. Many honeycombed cavities were found, which were separated from one another by a fine layer of connective tissue. There also were multiple gummas. Tauber believes that there was a primary syphilitic interstitial pneumonia with formation of gummas and resulting marked thickening of the septums of the lungs. Following the lobar pneumonia, abscesses had formed with destruction of some septums, whereas most of the other septums remained intact because they were markedly thickened as a result of the syphilitic process. Thus small and larger cavities were formed which gave a honeycomb-like picture. There were also syphilitic aortitis, gummas of the right testis and papilloma of the larynx.

OTTO SAPHIR.

ANTERIOR PROLAPSE OF THE INTERVERTEBRAL DISKS. W. HAMMERBECK, Virchows Arch. f. path. Anat. **294**:8, 1934.

In his systematic studies of the vertebral column Schmorl described a variety of conditions characterized by the presence of cartilaginous nodules in abnormal situations. They were seen most often in the cartilaginous plate or spongy bone of the vertebral body. Larger cartilaginous protrusions of the posterior margin of the disks were also described by Schmorl. These lie beneath the longitudinal posterior common ligament of the spine and protrude into the spinal canal. The formation of cartilage Schmorl held to be secondary to prolapse of the tissue of the nucleus pulposus, brought about by mechanical pressure on the disks. Hammerbeck describes protrusions of the anterior or anterolateral margins of the disks, beneath the anterior common ligament. These are also due to mechanical pressure. They are due to prolapse of nucleus pulposus tissue or, more often, of degenerated tissue of the annulus fibrosus.

O. T. SCHULTZ.

Microbiology and Parasitology

LONG-CONTINUED VACCINE THERAPY AS A CAUSE OF AMYLOIDOSIS. H. A. REIMANN and C. M. EKLUND, Am. J. M. Sc. **190**:88, 1935.

A patient suffering from chronic arthritis was given forty-one injections of vaccine over a period of twenty-two months. During this treatment amyloid disease developed, and the patient died. Because of the rarity of the occurrence of amyloid disease in chronic arthritis and the frequency with which it occurs following long continued parenteral injection of numerous substances including vaccine, it was believed that vaccine therapy was responsible for amyloidosis in this case, the first that we know of to be so described.

FROM THE AUTHORS' SUMMARY.

THE VIRUS OF LYMPHOGRANULOMA INGUINALE. R. D'AUNOY, E. VON HAAM and L. LICHTENSTEIN, Am. J. Path. **11**:737, 1935.

Seven endemic strains of the virus of lymphogranuloma inguinale have been isolated and transmitted to animals. Intracerebral inoculation of infectious material produced typical meningo-encephalitis in the common marmoset, while the rhesus monkey proved resistant to such inoculations. The virus could readily be transmitted to white mice, biweekly inoculation allowing upkeep of its maximal virulence. Emulsions of brain from infected monkeys and mice act excellently as stable and sensitive antigens for the specific diagnostic intradermal reaction of Frei. Twenty-eight per cent of infected guinea-pigs showed enlargement of the regional lymph glands with histologic lesions consistent with the disease. Experiments with sheep, chickens and frogs indicate that the virus can infect sheep, that its virulence can be preserved in the brains of chickens, and that frogs cannot be infected.

FROM THE AUTHORS' SUMMARY.

CORNEAL REACTIONS OF GUINEA PIGS TO TUBERCULO-PROTEIN AND TUBERCULO-PHOSPHATIDE. S. W. HOLLEY, Am. J. Path. **11**:937, 1935.

A study was made of tissue reactions to purified tuberculoprotein and tuberculophosphatide in the cornea of normal and tuberculous guinea-pigs. It was found that tuberculoprotein had a markedly toxic action on the connective tissue of the cornea of the tuberculous animal and led to inflammation and partial degeneration (tuberculin reaction). Furthermore, it seemed responsible, probably indirectly, for the production of epithelioid cells in the later stages of the allergic reaction. In the amounts used, the protein was practically inert in normal guinea-pigs. Tuberculophosphatide also caused an acute tuberculin-like reaction in tuberculous guinea-pigs. Inasmuch as the preparation contained a small amount of nitrogen, believed to be an impurity and not part of the molecule, it was concluded that the reaction noted was probably a reaction to tuberculoprotein. The fact that the unknown substance was closely bound chemically seemed to explain the fact that the acute reaction to the tuberculoprotein alone was diffuse, while that to the tuberculophosphatide was localized. In both tuberculous and normal animals epithelioid cells were present at the later periods and persisted longer than did those in most of the tuberculoprotein reactions. The observations confirmed previous work indicating that in tuberculosis of the cornea most mononuclears taking part in the reaction at the site of injection came from the blood stream, and that epithelioid cells arose from these mononuclears by a process of transition at the site of inflammation.

FROM THE AUTHOR'S SUMMARY.

OBSERVATIONS ON MULTIPLE TUBERCULOUS CALCIFICATIONS. C. SWEANY, Am. Rev. Tuberc. **32**:73, 1935.

A study of multiple calcifications selected from over six hundred persons examined post mortem and from various dispensary and hospital patients is reported. Calcifications may result from encapsulated tuberculous foci of any type, namely:

multiple primary seedings; scattered foci occurring in a resolving benign type of tuberculous pneumonia; hematogenous seedings occurring in the ante-allergic period of the secondary stage of the primary infection, various types transitional from the primary infection to the reinfection; hematogenous reinfections, and healing tuberculous nodules or infiltrates of any form arising from bronchogenic spread. Calcification may never occur at all in progressive tuberculous reinfection, and may occur only slightly or not at all in many massive primary infections. In both types, calcification seems to occur only in older lesions that have been well encapsulated. As the primary lesions become better encapsulated they have a great tendency to calcify. Nevertheless any secondary type of caseous lesions that ultimately heal will also calcify. The differences between the primary and the reinfection tubercles are reflected roughly in their calcified and ossified forms, which apparently change from a primary type to a secondary type, and gradually, with the development of allergy.

H. J. CORPER.

EPIDEMIOLOGICAL ASPECTS OF SILICOSIS AND TUBERCULOSIS. A. S. POPE and D. ZACKS, Am. Rev. Tuberc. **32**:229, 1935.

In representative groups of both granite workers and foundrymen in Massachusetts the frequency of silicosis and of silicosis and tuberculosis was correlated with the duration of exposure to dusts containing free silica and with the concentration of such dusts in the occupational environment. Among granite workers silicosis was found in 15.2 per cent, with tuberculosis in 7.6 per cent. Tuberculosis is the cause of death in over one third of all granite workers, a proportionate mortality three times that in foundry workers and four times that in all men 20 years of age and over. Among the employees of ten Massachusetts foundries, silicosis was found in 8.8 per cent, with tuberculosis in 2.6 per cent, a total frequency of one-half that in granite workers. The silicosis in foundrymen was not only less frequent but also less advanced in degree than that in the granite workers. Pneumonia is the greatest occupational hazard in the industry, causing one fourth of all the deaths of foundrymen.

H. J. CORPER.

INVOLVEMENT OF THE SPINAL MENINGES AND OF BONE IN UNDULANT FEVER SIMULATING TUBERCULOSIS. S. U. MARIETTA, Am. Rev. Tuberc. **32**:257, 1935.

The available literature on brucella infections involving the meninges and bones in man is reviewed. A description is given of a case that was probably one of brucella infection (bovine type) complicated with involvement of both the spinal meninges and the lumbosacral joint, with complete recovery after two years and three months. A positive diagnosis may be made in such a case, notwithstanding that cultures from the usual sources are negative, by a practical consideration of the history, clinical course and agglutination reactions and the elimination by various laboratory procedures, including inoculation of suspected material into animals, of the other possible etiologic factors.

H. J. CORPER.

THE FILTRABILITY OF TRACHOMA VIRUS. P. THYGESON and F. I. PROCTOR, Arch. Ophth. **13**:1018, 1935.

Inoculation of four baboons with bacteria-free filtrates of trachomatous materials resulted in a disease identical with that produced with unfiltered material. These experiments support the conclusions of Nicolle, Cuénod and Blaizot that trachoma is caused by a filtrable virus.

FROM THE AUTHORS' SUMMARY.

EXPERIMENTAL STUDIES ON THE PATHOGENESIS OF EPITUBERCULOSIS. E. H. OPPENHEIMER, Bull. Johns Hopkins Hosp. **57**:247, 1935.

A lesion, roentgenologically quite like that of so-called epituberculosis or the benign resolving pulmonary tuberculosis of childhood, was produced experimentally by introducing dead tubercle bacilli into the bronchus in hypersensitive rabbits. The shadow, which exhibited the form and clinical course of that in human epituberculosis, reached its maximum density in from two to four weeks, then gradually cleared completely, the clearing often beginning at the periphery, as is common in the shadow of epituberculosis. No similar shadow occurred when nonallergic animals were subjected to the same procedure. When immunized, hypersensitive animals were given intratracheal injections of living tubercle bacilli a similar shadow developed which, however, spread progressively to a fatal termination. The macroscopic appearance of the lesion with the epituberculosis-like shadow, studied at different intervals, was that of a tuberculous pneumonia which clears by resolution and organization. After resolution, strands of nonspecific scar tissue were found at the site in some cases. Histologically, the lesion produced was that of a typical tuberculous pneumonia with epithelioid and giant cells, lymphocytes and caseation. Tubercle bacilli or their fragments could be stained as long as the reaction remained. Later, peripheral organization, absorption of the exudate and nonspecific scar formation occurred. These observations demonstrate that the peculiar characteristics of epituberculosis can be reproduced in an allergic individual by the discharge of dead bacilli into the lung by way of a bronchus. On the basis of this fact, together with certain considerations discussed in the body of this paper, it is suggested that characteristic epituberculosis represents in many, if not in all, cases the result of erosion of a bronchus by a caseous lymph node containing relatively few viable bacilli, and that the characteristic shadow represents a tuberculous pneumonia produced by the discharge of caseous material impregnated with tuberculoprotein and dead bacilli, though containing some living bacilli, into the lung of a body sensitized and immunized by the previous infection. If a similar accident occurs in the case of a node teeming with live bacilli, a shadow of precisely similar form develops, but the process extends progressively. The various and clinically familiar shades between the benign retrogressive wedge-shaped area of epituberculosis and the wedge-shaped rapidly progressive area of active tuberculosis may be expected to occur depending on the balance between the number of viable bacilli in the discharging node and the degree of acquired resistance and hypersensitivity of the body.

FROM THE AUTHOR'S SUMMARY.

ACUTE STAPHYLOCOCCAL INFECTION OF THE JEJUNUM AND ILEUM. S. S. BLACKMAN JR., Bull. Johns Hopkins Hosp. **57**:289, 1935.

Blackman describes an example of staphylococcic infection of the small intestine which gave rise to septicemia and was fatal within approximately thirty-six hours after the onset of symptoms. The early symptoms were like those of staphylococcic food poisoning, and consisted of nausea, vomiting, diarrhea and prostration. Later, with septicemia and circulatory failure, coma and various other neurologic signs appeared. The autopsy showed widespread lesions of unusual character in the jejunum and upper portion of the ileum. The intestinal contents were blood-stained and included long molds of the mucosa, which were composed of necrotic fragments of intestinal villi, fibrinopurulent exudate and staphylococci in large numbers. The microscopic sections showed diffuse superficial necrosis and acute inflammation of the mucosa of the jejunum and of the affected portion of the ileum, associated with numerous staphylococci in the inflamed tissues. Thrombi were found in small vessels in a number of organs and were most numerous in the jejunum, ileum and liver. There were focal necrotic areas in the liver, spleen and bone marrow, and small hemorrhages, not associated with thrombi in blood vessels, were scattered in the brain and meninges. FROM THE AUTHOR'S SUMMARY.

AN EPIDEMIC OF TRICHINOSIS IN MAINE. E. H. DRAKE, R. S. HAWKES and M. WARREN, J. A. M. A. **105**:1340, 1935

The first epidemic of trichinosis to be recorded in Maine, so far as we have been able to determine, is the largest epidemic to be reported in the United States. Infection came from eating home-made pork sausages, improperly cooked. The infected persons, with a single exception, were Italians. Trichinellae were found in the infected meat and in the striated muscles of the two persons who died from the infection, and were examined post mortem. Seventy-one persons are known to have eaten the sausages, and fifty-six showed signs of infection. Twenty-six persons were ill with what was probably trichinosis; satisfactory skin tests were applied to twenty-four of these patients, and in all instances the intradermal reaction was positive. Two other persons, who gave a history of infection of the upper respiratory tract, showed eosinophilia but gave negative skin reactions. Twenty-five of the seventy who had eaten meat from the diseased hog gave no history of illness but were found to have eosinophilia; twelve of this group reacted to the skin test and thirteen did not.

Persons who eat small quantities of trichinous meat may present subsequent eosinophilia without definite illness; it is believed that these persons have latent cases of trichinosis. Barchman's intradermal reaction is positive in persons who are or who have recently been ill with trichinosis; the test may give negative results in those in whom the disease is latent.

FROM THE AUTHORS' SUMMARY AND CONCLUSIONS.

PATHOGENIC ORGANISMS OF THE GENUS LISTERELLA. C. V. SEASTONE, J. Exper. Med. **62**:203, 1935.

From persons with meningitis from cattle and sheep with encephalitis, from fowl with a myocardial infection and from rabbits with a generalized infection, different observers have isolated gram-positive organisms which are closely related. The cultural and serologic properties of the organisms are described. When the cultures are injected intravenously into chickens, rabbits or guinea-pigs there is an unusual blood response, the monocytes being markedly increased in number. The organisms tend to localize in the myocardium, with necrosis resulting.

FROM THE AUTHOR'S SUMMARY.

ISOLATION OF POLIOMYELITIS VIRUS FROM THE NASOPHARYNX. J. R. PAUL, J. D. TRASK and L. T. WEBSTER, J. Exper. Med. **62**:245, 1935.

A single example of mild illness which was suspected to be abortive poliomyelitis is described in which the virus of poliomyelitis was recovered from the nasopharynx by three different methods. Failure to recover the virus from twenty-six patients whose illness was suspected to be or was diagnosed as abortive poliomyelitis and from fourteen contacts is also reported. The original material from the nasopharynx of the patient in whose case examination gave positive results proved unusually infective for the monkey, apparently even more so than are the majority of suspensions of spinal cord from persons who have died of poliomyelitis. An explanation of this fact is not clear. The method of isolating human virus from the throat by preserving the sediment of washings from this site in glycerin has been shown to be efficient in one case for a period of one hundred and one days.

FROM THE AUTHORS' SUMMARY.

EPIDEMIOLOGY OF EQUINE ENCEPHALOMYELITIS IN THE EASTERN UNITED STATES. C. TENBROECK, E. W. HURST and E. TRAUB, J. Exper. Med. **62**:677, 1935.

Equine encephalomyelitis of the eastern type is a disease of the late summer and fall, and cases are found in greatest numbers near salt marshes. The epidemiological findings are against its transmission by contact, the view being favored that

it is insect-borne. Although virus can be demonstrated in the blood of infected horses it is present for a relatively short time, and the possibility that the disease is not primarily an infection of horses but is transmitted to them from another host is considered.

FROM THE AUTHORS' SUMMARY.

THE EFFECTS OF NASALLY INSTILLED VIRUS OF POLIOMYELITIS ON THE CEREBROSPINAL FLUID AND THE BLOOD OF MONKEYS. S. FLEXNER, J. Exper. Med. **62**:787, 1935.

Macacus rhesus and Macacus cynomolgus exhibit a striking sensitivity to the presence of the virus of poliomyelitis on the nasal mucous membranes. Irrespective of whether detectable symptoms of clinical poliomyelitis do or do not arise in the animals given nasal instillations, the cerebrospinal fluid changes quickly in response to virus placed in the nasal passages. Two sets of changes occur in the cerebrospinal fluid: a constant change, and the most pronounced one, is an increase in the fluid's content of white cells, chiefly of the lymphocytic type; an inconstant and less profound one is the appearance of detectable amounts of globulin in the fluid (free from red corpuscles) withdrawn by cisternal puncture. As early as forty-eight hours after instillation of the virus a marked increase in cells is detectable in the fluid; the increase grows from day to day, reaching a maximum sometimes in another day or two, sometimes not until four, five or six more days elapse. In many instances a rise of temperature follows or coincides with the rise in the number of cells, and the onset of clinical symptoms of the disease also bears a relation to the cell count. The number of cells in the cerebrospinal fluid and the temperature tend to be higher in monkeys in which paralytic symptoms develop; occasionally exceptions to this rule occur in which monkeys given instillations and remaining asymptomatic exhibit high cell counts; rarely do the latter show the higher temperatures. Monkeys that have once received instillations but failed to become symptomatically affected react again by cerebrospinal fluid changes to later courses of instillation. A second and still later course may induce paralysis, or highly exceptional or refractory animals may go through several courses of instillation without clinical symptoms developing, although they never fail to respond with changes in the cerebrospinal fluid. Instillations of virus into Macacus monkeys do not lead to active immunization unless clinical symptoms of infection result from the inoculations and attend the cerebrospinal fluid changes. In the complete absence of clinical symptoms in animals given instillations blood antiviral properties fail to develop, and these animals are as susceptible to cerebrally injected virus as are the control monkeys. On the other hand, monkeys which have been given instillations and which have shown even mild and fleeting (abortive) clinical symptoms of infection resist cerebral inoculation and exhibit blood antiviral or neutralizing properties. Monkeys which have shown clinical symptoms of the disease and have recovered are, as stated, actively immunized; they remain, however, sensitive to the presence of the virus on the nasal membrane, reacting with cerebrospinal fluid changes differing only in degree from those found in the nonimmune animals. Detectable virus does not appear in the pleocytic cerebrospinal fluid at any stage of the pathologic processes. The current belief is that mass immunization is proceeding in an unperceived manner through the chance entrance of virus into the nasal passages of children. It is not known whether, apart from all symptoms of the disease, cerebrospinal fluid changes occur in the course of this unexpressed spontaneous process. A large gap seems to exist between man and the monkey in the capacity of the former and the inability of the latter to become immunized by way of the nasal membrane. It is common knowledge that monkeys do not become immune through unsuccessful cerebral inoculations of virus, and the same seems to be true when the nasal passages are the channel for the penetration of virus into the central nervous organs.

FROM THE AUTHOR'S CONCLUSIONS.

Immunology

AN ATTEMPT TO "DESENSITIZE" TUBERCULOUS GUINEA PIGS WITH DEAD VACCINE AND PRODUCTS OF THE TUBERCLE BACILLUS. C. L. DERICK, E. A. G. BBANCH and M. P. CRANE, Am. Rev. Tuberc. **32**:218, 1935.

Subcutaneous vaccinations of tuberculous guinea-pigs with heat-killed tubercle bacilli resulted in quantitative diminution of the intracutaneous tuberculin reaction and prolongation of life over that of the control animals. Intravenous inoculation proved too fatal. No substantial results were obtained with formaldehyde-killed bacilli, Koch's bacillary emulsion or the protein fraction precipitable by acetic acid. The striking differences in the reactions of a sensitive human adult to an intracutaneous inoculation of unheated tuberculin and vaccination with killed tubercle bacilli are described. Whether any satisfactory antigen for active desensitization can be found remains to be determined.

H. J. CORPER.

ALLERGIC THEORY OF SO-CALLED THYMIC DEATH. G. L. WALDBOTT, J. A. M. A. **105**:657, 1935.

The evidence presented here suggests that the condition termed "thymic death" is a preallergic phenomenon similar to or identical with anaphylactic shock. It may be brought on by ingestion, inhalation, injection or absorption through the skin of antigens to which unusual sensitivity exists. In addition, sensitization to cold and heat, mechanical stimuli and infection should be considered as exciting agents.

SPECIFICITY IN MULTIPLE HYPERSENSITIVENESS. J. HARKAVY and E. WITEBSKY, J. Allergy **6**:437, 1935.

A review of these investigations indicates that in patients with multiple hypersensitiveness demonstration of corresponding reagins by the Prausnitz-Küstner technic gives only qualitative information as to the antibodies present. This method affords no insight in respect to the concentrations of the reagins in the serum or their independence or mutual interdependence (complete or partial); the latter may exist as a result of stimulation by biochemically related antigens. A true evaluation of such factors can be obtained only by both quantitative titration and absorption of the respective reagins. This method carried out in the study of the serums of our two patients, one of whom reacted to tobacco and ragweed, and the other of whom was shown with the ordinary passive transfer to have antibodies for various cereals, meats, fruits and vegetables, yielded the following information: Quantitative titration of the tobacco reagins in patient 1, who had coronary artery disease with angina pectoris associated with clinical hypersensitiveness to tobacco, disclosed that the upper limit of reaction was reached with a serum dilution of 1:100 and tobacco antigen (Virginia, 0.07 mg. of nitrogen per cubic centimeter) in a dilution of 1:100. This indicates a high degree of sensitivity to the tobacco extract employed. Previous addition of tobacco extract to the serum of this patient in vitro inhibited sensitization of normal skin on passive transfer, indicating absorption of the related antibody. In patient 2, whose condition was diagnosed as familial periodic paralysis and eczema, who displayed multiple sensitivity and had multiple reagins to timothy, corn, tobacco, etc., corresponding to his clinical symptoms, a study of these reagins yielded these data: Quantitative titration of reagins for timothy, tobacco and corn indicated that these were present in various concentrations. Preliminary addition of timothy extract to serum in vitro exhausted the reagin for timothy and corn but not that for tobacco. Previous addition of tobacco extract to the serum fixed the reagin for tobacco and corn but not that for timothy. Addition of corn extract to this serum absorbed the antibody for corn but did not influence that for tobacco or that for timothy. It may therefore be concluded that in the patients studied the major reagins were those for tobacco and timothy. These were specific and independent. Consequently the skin reactions were due to a reagin mechanism.

FROM THE AUTHORS' SUMMARY.

A Quantitative Theory of the Precipitin Reaction. M. Heidelberger and F. E. Kendall, J. Exper. Med. **62**:697, 1935.

A quantitative theory of the precipitin reaction based on the laws of classic chemistry has now been found applicable to the crystalline egg albumin-antibody system. Equations derived from the theory permit the calculation of the behavior of an anti-egg albumin serum over most of the reaction range after a few quantitative analyses have been made for the nitrogen precipitated. Data of other workers also conform to the proposed equations. The empirical relation, shown to have advantages in the dye-antidye system, may also be used for the egg albumin-antibody reaction. Serum from the same animal after successive courses exhibits progressive changes which have been described graphically and quantitatively. These changes are believed to consist in the formation of more and more antibody capable of reacting with a larger number of chemically different groupings in the antigen molecule. Evidence is presented that anti-egg albumin is not homogeneous, and that even after prolonged immunization the antiserum contains much low grade antibody, incapable of forming precipitates unless more reactive precipitin is present. Factors affecting the equivalence point ratio are discussed.

From the Authors' Summary.

Tumors

Myosarcoma of the Diaphragm. J. D. Kirshbaum, Am. J. Cancer **25**:730, 1935.

Two cases of sarcoma of the diaphragm with extensive metastases are described. One tumor could be identified as leiomyosarcoma. In the other tumor the character of the cells suggested a relation to striated muscle fibers. The two types of malignant tumors derived from skeletal muscle fibers are discussed: (1) the myoblastic sarcoma, which originates from undeveloped muscle fibers, the myoblasts; (2) the rhabdomyosarcoma, which is related to more mature striated muscle fibers. In contrast to the two malignant types there are the benign forms: the myoblast myoma and the rhabdomyoma. There are probably many undifferentiated myogenic tumors originating from striated muscle, diagnosed simply as sarcoma. Six cases of primary neoplasm of the diaphragm are cited from the literature, in three of which the growth was malignant From the Author's Conclusion.

Possible Effect on Survival Time of Oil of Gaultheria in Diet of Mice Susceptible to Spontaneous Carcinoma of the Breast. L. C. Strong, Am. J. Cancer **25**:797, 1935.

Mice that have received daily small amounts of the natural oil of wintergreen live longer than controls after the development of carcinoma of the mammary gland. The longer the mice have been on a diet containing wintergreen oil the longer they live after carcinoma develops. The earlier the age at which the mice are started on the diet the longer they live with their spontaneous tumors.

From the Author's Conclusions.

Cancer in Java and Sumatra. C. Bonne, Am. J. Cancer **25**:811, 1935.

Outstanding features of the pathology of tumors in Java and Sumatra are: 1. The extremely high incidence of primary cancer of the liver in native Malays and Chinese. This form of cancer shows in Netherlands India the highest incidence to be found in all statistics of autopsies. It is nearly always a carcinoma of liver cells, developing in a cirrhotic liver, cirrhosis of the liver of the Laënnec type and not due to parasites being prevalent among both races but especially among native Malay males.

2. The great rarity of gastric carcinoma among the native Malays but not among the Chinese, associated with a similar difference in frequency of gastric ulcer in the two races.

3. A certain frequency of oral carcinoma, especially of the cheek, in native men and women but not among the Chinese, which may or may not depend on the betel-chewing habit.

4. The prevalence of carcinoma of the lower parts of the legs in native Malay men, depending on the great number of ulcers present on the legs of these natives.

5. A peculiar frequency of malignant tumors of the cervical lymph nodes in Malays and Chinese, presenting a problem very complicated in its histologic aspects. Many of these tumors probably arise primarily from the reticulo-endothelial elements of these glands.

6. The rarity of carcinoma of the gallbladder, associated with infrequency of gallstones.

The most important of these peculiarities were pointed out by Snijders and Straub in 1921. They have been confirmed and substantiated by all subsequent researches on the subject. Similar problems present themselves in other parts of the Far East. The infrequency of gastric carcinoma and gastric ulcer, however, seems to be a special feature of the Malay race inhabiting Java and Sumatra and other islands of the Malay Archipelago. There is much need of a comparative statistical investigation of autopsy records in various countries of the Far East with regard to the frequency and the site of cancer.

FROM THE AUTHOR'S SUMMARY.

MULTIPLE MYELOMA WITH HYPERPROTEINEMIA. C. F. SWEIGERT, Am. J. M. Sc. **190**:245, 1935.

A condition diagnosed clinically as multiple myeloma with hyperproteinemia is described and the cases in the literature reviewed. Hyperproteinemia is decidedly uncommon and occurs in its most striking form in multiple myeloma. In the majority of instances it has been attributable to hyperglobulinemia without demonstrable relationship to Bence-Jones protein. In three cases it was due to extraordinary amounts of Bence-Jones protein in the serum. In the present case, both the globulin and the fibrinogen in the blood plasma were increased. Hyperproteinemia may produce unusual and variable clinical phenomena, notably: difficulty in counting erythrocytes; autohemagglutination; markedly accelerated sedimentation of the erythrocytes; abnormal coagulability of the blood, and spontaneous precipitation of protein in drawn blood. In the presence of Bence-Jones proteinemia, precipitation in the serum may occur during inactivation for the Wassermann reaction. The finding of hyperproteinemia or any of its manifestations should suggest multiple myeloma as a diagnostic possibility. The clinical aspects of the blood and renal changes in the present case are emphasized and their diagnostic importance indicated. The changes include (*a*) a severe, progressive macrocytic anemia with evidences of profound disturbance and active regeneration of the bone marrow, unassociated with achlorhydria and refractory to liver and iron therapy, and (*b*) an atypical nephropathy in which impairment of renal function without hypertension is a distinctive feature. FROM THE AUTHOR'S SUMMARY.

THE CUTANEOUS GLOMUS AND ITS TUMOR—GLOMANGIOMA. O. T. BAILEY, Am. J. Path. **11**:915, 1935.

The cutaneous glomus is an arteriovenous anastomosis in the stratum reticulare of the cutis, which is homologous with the glomus coccygeum and several less important vascular structures. These have in common a specialized cell, which is a modified smooth muscle cell with abundant nervous connections. The cutaneous glomus has an important function as an arteriovenous shunt in maintaining the body temperature and perhaps the blood pressure. From the cutaneous glomus a tumor may arise which forms a subgroup of the hemangioma. The term "glomangioma" is suggested for it to indicate its derivation and character. Glomangioma appears as a small bluish nodule on an extremity or an adjacent portion of the

shoulder girdle. Very frequently it is located in the nail bed. Microscopically the tumor is composed of cells identical with those in the walls of the normal cutaneous glomus and its homologs. Nerve trunks are numerous in the connective tissue about the tumor, and nerve filaments pass among the glomus cells in large numbers. Occasionally elongate smooth muscle cells are seen either in solid masses or adjacent to vascular lumens. The glomangioma represents the overgrowth of the entire arteriovenous anastomosis, and in doing so the cells show a twofold differentiation: First, the elongate smooth muscle cells lose all myofibrils, while the reticulum investing them becomes much coarser and stains intensely with collagen stains. Second, the periglomic nerves grow into the tumor and their terminal filaments end about the differentiating smooth muscle cells with the interposition of nerve endings. These two processes result in the formation of the glomus cells and are apparently interdependent. The tumor is associated clinically with severe radiating pain of neuralgic type. In character and distribution this has many similarities to the response of the normal glomus to much greater stimuli of the same character. Glomangiomas thus represent functionally as well as morphologically organoid overgrowths. Glomangiomas do not become malignant. Local excision gives complete and permanent relief from symptoms. FROM THE AUTHOR'S SUMMARY.

MALIGNANT MONOBLASTOMA: A VARIANT OF MONOCYTIC LEUKEMIA. L. A. MITCHELL, Ann. Int. Med. **8**:1387, 1935.

Mitchell states that although forty-two instances of monocytic leukemia have been reported in the literature, none of them exhibited extensive tumor formation with primary multiple monoblastoma of the connective tissues with a terminal leukemic phase in which a large number of primitive monoblasts flooded the circulation. The instance he records occurred in a railroad engineer, aged 63, who complained of abdominal distention and reddish eruptions on the inner surfaces of the calves of the legs and was seen to have small subcutaneous nodules (200) distributed over the trunk, arms and upper thirds of the thighs. The nodules responded to roentgen therapy, only to recur with increasing numbers three months later. The blood leukocytes varied in number from 1,500 to 5,000 and the monocytes from 0 to 1,300 during the aleukemic phase, while there was a variation of from 3,000 to 130,000 and of from 3,880 to 127,400 respectively, in the leukemic phase. At autopsy, nodules and diffuse infiltrations were found in the tissues generally. Microscopic sections (biopsy and postmortem) disclosed typical monoblasts with multinucleated giant cells only in the liver, kidney and spleen, where reticular hyperplasia was also noted. FRANK R. MENNE.

PINEALOCYTOMA WITH MENINGEAL AND NEURAL METASTASES, E. D. FRIEDMAN and A. PLAUT, Arch. Neurol. & Psychiat. **33**:1324, 1935.

A small tumor of the pineal body is described which caused no local signs and symptoms but produced the picture of a disseminated lesion of the nervous system. The patient, a man aged 33, presented first an infundibular syndrome which, in the course of time, became complicated by a bilateral paralysis of the third nerve, optic atrophy, irregular anesthesias of the lower extremities, absence of tendon reflexes and xanthochromia of the spinal fluid with an immense pleocytosis and increased protein content. Mental symptoms (euphoria) developed, and encephalography revealed hydrocephalus. The necropsy showed thickening of the cranial nerves, especially of the third, fifth and seventh, and of the meninges, the spinal ganglions and the roots of the cauda equina. The thickening was due to infiltrations by small cells the size of lymphocytes which metastasized from a pinealocytoma. As the tumor cells obstructed the perineurial spaces of the cerebral (except the optic and olfactory) and spinal nerves—the avenues of absorption of the cerebrospinal fluid—the authors consider the obstruction as the cause of the hydrocephalus. The parenchyma of the brain and cord was free from tumor cells except in the perivascular spaces of the peripheral portions. GEORGE B. HASSIN.

Medicolegal Pathology

THE MICROSCOPIC EXAMINATION OF THE PULMONARY ALVEOLI IN BURNT BODIES. A. FOERSTER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **23**:281, 1934.

Burning during life may result in destruction of the elastic fibers, widening of the septums and collapse of parts of the lung. Even postmortem burning may cause changes in the elastic fibers in the lungs but only at the place of action of the heat. The changes may be regarded as vital when they are evenly distributed throughout the lungs. When the bodies of newly born infants are burnt it may happen that under the direct influence of the high temperature aerated lungs assume an atelectatic appearance.

EXTERNAL EVIDENCE OF HANGING. K. WALCHER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **25**:141, 1935.

Three parallel abrasions of the skin of the under-surface of the chin were noted anterior to the furrow of the neck produced by the fatal noose. These were thought to be due to the rope scuffing the skin as it drew tighter in several stages from the weight of the body. The theory was proved experimentally, and lack of blood beneath such regions was seen to be due to the pressure of the rope.

GEORGE RUKSTINAT.

ALIVE OR DEAD WHEN HIT BY A RAILROAD TRAIN? H. MOHR, Deutsche Ztschr. f. d. ges. gerichtl. Med. **25**:147, 1935.

Three corpses badly mangled by railroad trains were studied in an attempt to ascertain whether the injuries were responsible for death or whether the bodies had been laid on the track in order to multilate them and divert suspicion of murder. In each of the bodies the head had been severed from the trunk and the organs of the neck torn off deep in the thorax. Extensive fractures were found, but fat emboli were lacking. Two bodies were practically bloodless, but tears of the spinal ligaments and muscles with hemorrhage and fractures of the vertebral bodies from overstretching the spinal column indicated the deaths were due to the railroad accident. The deaths were so quick that there was no reaction to the hemorrhage in the injured tissues. It was concluded that these observations concerned cases of suicide.

GEORGE RUKSTINAT.

INDELIBLE PENCIL POISONING. J. GIERLICH, Deutsche Ztschr. f. d. ges. gerichtl. Med. **25**:156, 1935.

Fatal poisoning resulted from the oral administration of the lead of an indelible pencil to a premature infant. The dye used to color the lead was methyl violet, a chemical occasionally reported as responsible for poisoning but not poisoning leading to death. The alterations in the infant's body suggested aniline poisoning.

Investigations with an aqueous solution of methyl violet were carried out on guinea-pigs and the following deductions made:

Methyl violet causes severe necrosis of tissue at the site of injection. From this site the dye is transferred in a colorless form in the blood stream. It is a strong protoplasmic poison which destroys red blood cells with the production of anemia and the formation of methemoglobin. In the young warm-blooded animal there is only a poorly developed ability to detoxify methyl violet; in the adult this ability is more adequate. The histologic picture resembles that of aniline poisoning.

GEORGE RUKSTINAT.

THE DEMONSTRATION OF THALLIUM IN THE TISSUES. J. SCHNELLER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **25**:222, 1935.

Thallium was demonstrated spectroscopically in the urine and stool of a woman three and one-half months after she had ingested 0.37 Gm. of the poison. It could not be detected by ordinary means. This slow excretion was confirmed in dogs and guinea-pigs poisoned with thallium. Tests showed its presence in the bones long after all traces of the metal had disappeared from other organs.

GEORGE RUKSTINAT.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

PERCIVAL BAILEY, *President*

Regular Monthly Meeting, Jan. 13, 1936

EDWIN F. HIRSCH, *Secretary*

INFLUENCE OF LOCAL IMMUNIZATION OF THE LUNGS OF GUINEA-PIGS ON INTRATRACHEAL INFECTION WITH BACILLUS TUBERCULOSIS H37. R. L. FERGUSON and PAUL R. CANNON.

One hundred and thirty-seven guinea-pigs were used in a study of the influence of local immunization of the lungs against experimental tuberculosis. The animals were immunized intratracheally with a phenol-killed culture of Bacillus tuberculosis H-37 and were infected intratracheally some weeks later with living organisms of the same strain.

Intratracheal immunization conferred a definite increase of resistance. The treated animals lived almost twice as long as the controls similarly infected; there was less widespread dissemination of tubercle bacilli throughout the body, and the lesions in the vaccinated animals regressed more rapidly.

The local stimulation of the lungs favored mobilization of the macrophages in the interstitial tissues and in the regional lymph nodes so that when living tubercle bacilli traversed the same routes they were more effectively engulfed and destroyed. Fewer bacilli reached the blood stream, and fewer lesions occurred in the liver and spleen.

The experiments support the idea that direct antigenic stimulation of a portal of entry may cause a considerable increase in resistance against infectious agents which enter the body by the same route.

DISCUSSION

I. DAVIDSOHN: Were there differences in the allergic or complement-fixation reactions in the two groups of animals? Did the immunized animals lose weight?

H. C. SWEANY: Every one studying tuberculosis of human lungs clinically observes that tissues with healed lesions are rarely reinfected. If the lungs are reinfected, the disease develops in a portion that has not been involved. This indicates local resistance to infection. In the lungs of Dr. Cannon's untreated animals there were many bacilli; in those of the treated animals there were few. These results suggest another method of immunization against tuberculosis.

P. R. CANNON: Allergic and immune reactions were not studied. The immunized animals at first gained weight; the others lost weight rapidly. Immunization of animals with BCG by this method should be tried.

PATHOGENESIS OF TUBERCULOUS MENINGITIS. ALEXANDER B. RAGINS.

A study of brains from forty-seven patients, thirty-nine of whom had died primarily from tuberculous leptomeningitis, was made to determine whether the leptomeningitis was due to a local caseating and discharging tuberculous focus in the brain, meninges or adjacent bony structures or to a direct hematogenous dissemination of the bacilli to the meninges. The brains were cut in very thin slices (3 mm.) and studied both macroscopically and microscopically. Of the patients dying from tuberculous leptomeningitis, twenty-three, or 58.97 per cent, were

definitely free from older lesions of the brain, meninges or adjacent bony structures. Twelve, or 30.76 per cent, had multiple tuberculous foci; in only five of these did the foci seem to have been responsible for the tuberculous leptomeningitis. In the remaining seven patients the meningitis was apparently the result of direct hematogenous dissemination. In four, or 10.25 per cent, of the patients with tuberculous leptomeningitis the brains contained single older tuberculous foci; only in two of these could the leptomeningitis be attributed to the older foci. Eight of the patients did not have tuberculous leptomeningitis, and four of these had large tuberculous foci in the brain tissue.

In thirty-two, or 82.05 per cent, of the patients with tuberculous meningitis the meninges seemed to have been infected by hematogenous dissemination of the tubercle bacilli, and only in seven, or 17.94 per cent, did the meningitis appear to have developed by extension from older lesions in the brain or meninges.

DISCUSSION

A. WEIL: What histologic criteria do you use in determining the age of such tuberculous lesions?

P. STEINER: Some patients with generalized miliary tuberculosis do not have tubercles in their brains, and many patients with miliary tuberculosis do not have tuberculous meningitis. Tuberculous meningitis may occur also without miliary tuberculosis. In patients at the Children's Memorial Hospital below the age of 12 years, tuberculous meningitis has invariably accompanied systemic infections except in those with adult lesions. Tuberculous meningitis seems to follow hematogenous dissemination.

OTTO SAPHIR: Did serial sections demonstrate a spread of tuberculosis to the meninges from a solitary tubercle of the brain when a single section did not?

A. B. RAGINS: It is true that the age of a tubercle is difficult to determine by histologic examination. There are a great many factors to consider, among which are the resistance of the patient and the pathogenicity of the organism. Serial sections were not made.

OSSIFYING FIBROMA OF THE JAW BONES. KEITH GRIMSON.

The maxilla and the mandible ossify in membrane with only accessory incorporation of cartilage. This observation may be related to the fact that a benign cartilaginous tumor of either of these bones is seldom found. In one patient a cartilaginous exostosis developed in the coronoid process of the mandible. In this region of the human jaw enchondral ossification is present.

In the majority of cases a benign bony tumor of the jaw is an osteoma. Another patient had such a tumor in the midline of the palate. It was well localized and closely bordered by normal bone.

A third type of benign bone-containing tumor of the jaw characterized by an expansive enlargement of the bone and showing a mosaic of bone trabeculae and fibrous marrow spaces has been recognized since the first description by Menzel in 1872. Three cases are reported: In one patient a tumor of this type developed in the right maxilla at the age of 4½ years and one in the right mandible at the age of 10. In a second patient a tumor of the same type developed in the right maxilla at the age of 13, and in a third patient one developed in the anterior portion of the mandible at the age of 15.

A review of thirty-three reported cases has revealed the following terminology: "ossifying fibroma," "intraosseous epulis," "localized Paget's disease," "osteitis fibrosa localisata" and "osteodystrophia localisata." Since all of my three patients had normal concentrations of calcium and phosphorus in the blood, no cysts, and very few giant cells in the tumors, these lesions should be designated as ossifying fibroma. There were no recurrences following surgical removal with subsequent application of roentgen radiation in two cases.

HETEROGENOUS PRIMARY MALIGNANT TUMORS IN ONE HOST: HYPERNEPHROMA OF THE KIDNEY AND CARCINOMA OF THE PROSTATE. HAROLD R. OSTRANDER.

The right kidney removed from a white man aged 61 years weighed, with attached fat tissues, 149.5 Gm. In the lower pole was a hypernephroma measuring 5 by 4.5 by 3.5 cm. Fourteen days after the nephrectomy the prostate tissues were removed by transurethral electroresection. These weighed 4.3 Gm., and considerable portions contained glandular carcinoma the structure of which was that of a growth primary in the prostate. The man died at St. Luke's Hospital about twenty-eight months after the onset of renal symptoms and ten months after right nephrectomy and transurethral resection of the prostate. The postmortem examination (Edwin F. Hirsch) demonstrated thrombosis of the inferior vena cava and hepatic veins by hypernephroma, metastases of hypernephroma in the left kidney and the pancreas, and carcinoma of the prostate.

Bugher (*Am. J. Cancer* **21**:807, 1934) reported a like combination of malignant growths in one host. There seems to be no other description of one.

PERCIVAL BAILEY, *President*

Regular Monthly Meeting, Feb. 10, 1936

EDWIN F. HIRSCH, *Secretary*

OSTEOCHONDROMA OF THE RIB. D. B. PHEMISTER.

This cartilaginous tumor occurs on the rib either singly or as a part of multiple cartilaginous exostosis. It is usually located near the costochondral junction. In some instances the tumor is in the form of a cartilage-capped exostosis, which is small and resembles the exostosis that occurs on a long bone. In other cases a globular tumor either incorporates or completely replaces a segment of the rib and bulges both externally and into the thoracic cavity. The osteochondroma consists of hyaline cartilage, calcified cartilage and bone. One such tumor of the left eleventh rib, 14 by 10.5 cm., occurring singly, was reported in a 19 year old girl. The tumor had a peripheral layer of hyaline cartilage, but the interior was a jumbled mixture of hyaline and calcified cartilage with a small amount of bone. Another tumor was reported, 11 by 9 cm., involving the left third rib removed from a 27 year old woman who had multiple cartilaginous exostosis. In this tumor the hyaline cartilage, calcified cartilage and bone had much the same arrangement as in a growing epiphyseal line. The peripheral zone consisted of hyaline cartilage, the middle zone of calcified cartilage and the center of spongy bone with ossification progressing toward the periphery. This type is more likely to give rise to chondrosarcoma than the other type of cartilaginous exostosis. I have seen four examples of chondrosarcoma of this region.

ACTIVE AND PASSIVE IMMUNITY TO EXPERIMENTAL POLIOMYELITIS. F. B. GORDON.

Three different preparations of the virus of poliomyelitis were studied for their ability to induce immunity in the rhesus monkey. Virus adsorbed to a gel containing aluminum oxide was used because of the evidence from various sources that the antigenicity of a substance may be increased by adsorption to a colloid. An eluate from the virus-gel complex and an emulsion of the crude virus were used for comparison. From one to five subcutaneous injections were given to each monkey. Three monkeys were attacked by poliomyelitis as a result of the immunizing injections, one in the group treated with the virus-gel and two in the group treated with the crude virus.

Response to the antigens was ascertained in two ways: Each monkey was given an intranasal inoculation and his serum subjected to the in vitro neutrali-

zation test. Of nineteen monkeys in which both tests were completed, twelve had serum which neutralized the intranasal virus. Of these twelve, only two resisted intranasal inoculation. None of the seven whose serums failed to neutralize the virus resisted intranasal inoculation. A definite conclusion cannot be drawn regarding the efficiency of the virus gel as an immunizing agent. However, the individual tests as well as a test of pooled serums suggest that the virus gel elicits a slightly greater response than the crude virus.

Fifty-five test monkeys and forty-four controls were used in testing immune serums for in vivo protective properties. The serums were injected before and after administration of the virus by the intracerebral, the intravenous or the intranasal route. Transfusions of blood from hyperimmunized monkeys were also tried. The results of some experiments seemed to indicate that both the serum and the blood protected against an attack, but the results were not constant for any given technic and could not be obtained repeatedly. Sixty-five per cent of the test monkeys and 70 per cent of the controls had poliomyelitis.

Thus, the presence of neutralizing antibody in the serum of the blood stream has been induced by active injection of virus and by passive transfer of serum and blood from immunized animals. That the presence of antibody thus induced does not protect the monkey against a test dose of virus is indicated by the results given.

An explanation for this lies in the pathogenesis of the disease. Experimental evidence shows that the virus remains in intimate association with the cells of nervous tissue in its journey from the portal of entry, the nasopharynx, to the motor cells of the cord. Such an association may suffice to prevent any contact of virus with antibody.

It is suggested that both active and passive immunization, to be effective, must be directed either to the central nervous tissue or to the portal of entry.

DISCUSSION

I. DAVIDSOHN: The question as regards the nature of the antibodies in normal serum is important. Probably they are not significant as an immunizing factor. Many human serums have these substances, but how about monkey serums?

P. R. CANNON: How many infective doses were given in the intranasal injection? Is there any evidence of an immune effect with human serum?

F. B. GORDON: The presence of neutralizing antisubstances in the serum of normal monkeys has been studied by Jungeblut, who has reported finding them in adult animals only. He regards the presence of these antibodies as due not to a chance exposure but to normal physiologic maturation, whether hormonal or endocrine. Hudson and Lennette have been unable to confirm these observations in young and old monkeys of either sex or in pregnant ones. Human neutralizing serums were given to monkeys before and after the virus was injected, without effects. Highly potent neutralizing animal serums, e. g., horse serum, have had protective property when given before the virus. Attempts to determine a minimal infective dose of the virus have been unsuccessful. Others have claimed to use one, two or three minimal lethal doses, but I doubt that this can be done. The intranasal method of inoculation is not adapted to such a standardization, because an indeterminate quantity of the material containing the virus drops into the throat. However, the amount of the material sprayed into the nose was large. To what extent these results can be applied to human infections is difficult to determine. If the additional immunization can be shown to protect the nasal passages against infection, these results in monkeys do not hold for man. Human convalescent serum was for many years thought to be protective. That it is protective is doubtful. During the 1931 epidemic in New York convalescent human serum was used in treating the disease; in the recent Los Angeles epidemic it was used in prophylaxis. The results in both epidemics indicate that the use of convalescent serum before or during the disease has no value.

AN ANTIGENIC POLYSACCHARIDE FROM PORCINE ASCARIS LUMBRICOIDES. DAN H. CAMPBELL.

Remarkable advances have been made within recent years in immunochemical studies of carbohydrates. Investigators have been chiefly interested, however, in bacteria and related organisms. I undertook to determine the immunologic significance of the polysaccharides of parasites of animals. The present report is concerned with the study of the common porcine ascarid (Ascaris lumbricoides).

The polysaccharide was isolated by extraction of finely ground whole worm material with neutral aqueous solutions or by digestion of the proteins with pepsin at p_H 4.5. Further purification was carried out by removal of all nitrogenous material with phosphotungstic acid. Chemical analyses showed no nitrogen in 10 Gm. samples, and only hexoses, presumably dextrose, were found in the acid hydrolysate.

This substance in fairly high dilutions gave specific precipitates with antiserum against the whole worm material and gave positive skin reactions in rabbits sensitized with whole worm material. It also induced the formation of antibodies (precipitins) when injected intraperitoneally or subcutaneously into rabbits and in amounts of 0.00001 Gm. sensitized guinea-pigs so that subsequent injections gave positive anaphylactic reactions. Although a slight cross-reaction occurred between the polysaccharide and protein fractions, evidenced only in anaphylactic tests, a quantitative study of the reaction indicated that it was due probably to a detectable amount of polysaccharide in the protein fraction rather than to any possible protein contaminant of the polysaccharide fraction.

The ability of the polysaccharide to induce the formation of specific antibodies or to react with them was not altered by boiling in neutral solutions or by exposure to proteolytic enzymes. The ability to produce antibodies was, however, slightly decreased by exposure to p_H 4.5 for two days and completely destroyed by boiling in ten-thousandth-normal sodium hydroxide. These procedures had no effect on the capacity to react with specific antibodies. Boiling in hundredth-normal sodium hydroxide, however, completely destroyed the immunologic activity of the polysaccharide.

DISCUSSION

I. DAVIDSOHN: If this study should finally demonstrate that carbohydrates are antigenic, it would be the first to show that proteins are not the only substances capable of stimulating the production of immune bodies. Perhaps a small trace of nitrogenous substance, within the limits of error, is the real antigen.

P. R. CANNON: What is the yield of carbohydrate from the ascaris material?

D. H. CAMPBELL: The tests for nitrogen were made with quantities of carbohydrate material ranging from 1 to 10 Gm., using the macro-Kjeldahl method by which 0.2 mg. of nitrogen may be detected readily. The yield varies. With aqueous extraction it is 1 per cent; with the pepsin digestion, 2 per cent, and with boiling, from 3 to 4 per cent. The actual amount in fresh moist tissues is about 4 per cent.

MENINGOCOCCIC MYOCARDITIS. OTTO SAPHIR.

Myocarditis due to infection with meningococci is rare if one may judge from the literature. Practically only two published contributions as to the occurrence of isolated meningococcic myocarditis exist (Westenhoeffer and Gruber). There are, however, in the literature a number of reports of instances of meningococcic endocarditis. In these reports, lesions in the myocardium were merely mentioned and apparently were not considered to be significant. The observations presented here were made on two cases of meningococcic meningitis. In one instance signs of meningitis developed, and the patient died showing marked cyanosis about fifty hours after the onset of the disease. The second patient also had typical symptoms of meningitis, showed apparent improvement and was getting along seemingly well when cyanosis developed and she died. In both instances, the meningitis was evident

grossly at autopsy. The heart on gross inspection showed no abnormalities with the exception of cloudy swelling and dilatation of the ventricles. There was no endocarditis. In the first case, both the blood and the spinal fluid remained culturally sterile. Smears from the cerebrospinal fluid, however, showed the typical gram-negative intracellular diplococci. A pure growth of meningococci was obtained from the cerebrospinal fluid in the first instance.

Histologically myocarditis was found in both hearts. It was characterized by a hemorrhagic exudate, the early appearance of large endothelial cells, the destruction of muscle fibers and the presence of gram-negative intracellular biscuit-shaped diplococci. There also were foci of necrosis which were similar to those seen in the myocardium in instances of subacute bacterial endocarditis.

In view of the prevailing opinion that the primary condition in meningococcic meningitis is meningococcic bacteremia it is not surprising that occasionally meningococci may cause isolated myoarditis.

Book Reviews

Angiostomie und Organestoffwechsel. By Prof. Dr. E. S. London, All-Union Institut für experimentelle Medizin zu Leningrad. Paper. Pp. 193, with 54 illustrations. Moscow: Verlag des all-Union-Instituts für experimentelle Medizin, 1935.

The first fifty pages of this well written monograph describe, in minute detail, the technical procedure involved in what London has called "Angiostomy." The work was done on dogs and has made possible the sampling of blood from afferent or efferent blood vessels of organs ordinarily inaccessible in healthy unanesthetized animals. The technic consists essentially of a preliminary operation or operations in which one end of a long slender metal cannula is sewed to the outer wall of the blood vessel in question, while the other end of the cannula is allowed to protrude to the exterior through a stab wound at the appropriate site. When the animal has recovered from the operation, when the external wounds are healed and when the proximal end of the cannula is firmly attached to the blood vessel by fibrous tissue, the cannula acts as a guide for a long flexible hypodermic needle. This needle is inserted through the cannula, fibrous tissue and wall of the blood vessel each time a sample of blood is desired. The mechanical difficulties which must be overcome in working with the blood vessels of various organs are admirably described and are illustrated in an appendix of fifty-four figures.

The remainder of the volume summarizes the author's observations, by means of this ingenious method, on the various metabolic functions of individual organs, including the liver, spleen, intestine, kidney, brain and skeletal muscle. The problems investigated include phases of carbohydrate, protein, fat and mineral metabolism. The results of the experiments are given in many tables and are briefly described and discussed in the text. Bibliographic references are not numerous and are largely confined to the European literature.

To obtain his data London has analyzed simultaneously drawn samples of arterial and venous blood from a particular organ or organs. The arteriovenous difference in the amount of the material for which the analysis is made is taken to represent the extent to which the organ is either using or producing the material, depending on whether the difference is a positive or negative quantity. It is obvious that this method is suitable and valuable for determining, in a qualitative manner, whether a certain material is being retained or put out by an organ. But it seems equally clear that, unless the mass of the organ and the rate of blood flow through it are taken into account, the arteriovenous differences can have no quantitative significance. Unfortunately, the method does not permit the quantification of these essential factors. It is not surprising, therefore, that when the author attempts to compare the metabolic activity of different organs of different mass and with different rates of blood flow and under conditions in which the experimental procedure itself may change the blood supply, he sometimes derives bizarre conclusions.

This monograph will be of interest to some investigators for its careful description of a valuable technic and for that portion of its content which is factual in nature. It cannot be recommended to those interested in the less technical and more theoretical aspects of metabolism.

Manuel de Coprologie Clinique. By R. Goiffon. Third edition. Price, 28 francs. Pp. 274, with 45 illustrations. Paris: Masson & Cie, 1935.

The manual is divided into four parts: the physiologic processes concerned in the formation of feces, the examination of the feces, the evaluation of the results of fecal examination and therapeutic considerations. The book, which is in its third

edition, is a competent guide to the study of feces for clinical purposes. It contains full directions for the chemical, physical, parasitologic and microbic methods of examination. In the next edition the directions for the examination of feces for Endamoeba histolytica should be enlarged to include the newer methods.

A B C of the Endocrines. By Jennie Gregory, M.S. Foreword by Carl G. Hartman, Department of Embryology, Carnegie Institution of Washington. Price, $3. Pp. 126. Baltimore: Williams & Wilkins Company, 1935.

The object of this book is to provide a concrete summary of the knowledge of the functions of the endocrine glands. The method of presentation is original. The story of each gland and its relationships is told wholly by means of charts and graphs, with brief explanations in direct, sometimes telegraphic, style. There are ten chapters, and the following are discussed: the history of the glands, experimental methods and the pituitary, gonads, thyroid, adrenals, pancreas, parathyroids, pineal and thymus and other principles and glandular relationships. In each chapter the charts pass logically from the simple to the more complex situations. At the end are a select bibliography and a useful glossary with French, German and Italian equivalents. The charts and graphs are well done. The concrete information that they convey in a novel form appears to be trustworthy. Of course, further investigation may change conceptions that now appear well founded. The book will be of service to intelligent persons who are interested in reviewing the present state of knowledge of endocrine functions. Unfortunately, there is no title on the back of the book.

Atlas of Pathological Anatomy. Volume II. Issued under the direction of the editorial committee of the British Journal of Surgery. Compiled by E. K. Martin, M.S., F.R.C.S. Price, $15. Pp. 475. Baltimore: William Wood & Company, 1935.

The first volume of this atlas, which is a reprint of supplements to the *British Journal of Surgery*, was reviewed in the ARCHIVES (**17**:450, 1934). The present volume, which begins with supplement 21, illustrates mainly the gross appearance in certain diseases of the joints, thyroid, tongue, pharynx, esophagus, intestine, kidney, bladder and male genital organs and fibrocystic disease of bone. There are only occasional photomicrographs. The morbid anatomy of the organs mentioned is not covered systematically. On the whole, cancers predominate. The text consists of a brief description of the specimens illustrated, with a concrete clinical summary. The atlas will interest surgeons particularly. The drawings and the reproductions are uniformly excellent.

A Bibliography of Two Oxford Physiologists: Richard Lower, 1631-1691; John Mayow, 1643-1679. By John Fulton. Pp. 62. Vol. IV, Part 1. Reprinted from the Oxford Bibliographical Society Proceedings and Papers. Oxford: University Press, 1935.

This is a catalog, with a running critical and historical commentary, of works of two authors, which mark the development of experimental physiology at Oxford. Such a bibliography as this deserves to be read rather than merely to serve for reference purposes. One learns that Lower, who also was an eminent practitioner, made great forward strides in elucidating pulmonary circulation and that (in 1669) he explained several obscure points in the anatomy of the heart, in the origin and mechanism of catarrhal discharges and in the medical use of barley water. Mayow devoted himself to the study of respiration and the pathology and management of rickets. His work is intimately interwoven with that of Robert Boyle and Robert Hooke, and his chief scientific discovery seems to be that what is called hydrogen and nitrous oxide expand in the same way as air, or that they follow the law of Boyle.

Both bibliographies are accompanied by an elaborate critical apparatus and include biographic material of interest to the historical-minded student.

Books Received

THE BACTERIOLOGICAL GRADING OF MILK. G. S. Wilson, assisted by R. S. Twigg, R. C. Wright, C. B. Hendry, M. P. Cowell and I. Maier. Medical Research Council Special, Report Series, no. 206. Price, 7 shillings, sixpence. Pp. 392, with 29 illustrations. London: His Majesty's Stationery Office, 1935.

LE DIABÈTE SUCRÉ: QUESTIONS CONTROVERSÉES DE CLINIQUE ET DE PATHOGÉNIE. Leçons professées à l'Hôpital Saint-André de Bordeaux (Service du Professeur Mauriac). E. Aubertin, E. Bessière, P. Broustet, O. Hirsch, P. Mauriac, R. Saric, M. Traissac and F. J. Traissac. Price, 32 francs. Pp. 214. Paris: Masson & Cie, 1936.

LESIONS DU PANCRÉAS ET TROUBLES FONCTIONNELS PANCRÉATIQUES. DIAGNOSTIC EN CLINIQUE PAR L'ÉPREUVE À LA SÉCRÉTINE PURIFIÉE. Marc Bolgert, Ancien Interne, Lauréat des hôpitaux de Paris, Aide Préparateur au Laboratoire d'anatomie pathologique de la Faculté de Médecine. With a preface by Professeur Roussy. Price, 45 francs. Pp. 262, with 22 illustrations. Paris: Masson & Cie, 1936.

DELAFIELD AND PRUDDEN'S TEXT-BOOK OF PATHOLOGY. Revised by Francis Carter Wood, M.D., Director of the Pathological Department, St. Luke's Hospital, New York; Director of the Institute of Cancer Research, Columbia University, New York. Sixteenth edition. Price, $10. Pp. 1,406, with 861 illustrations. Baltimore: William Wood & Company, 1936.

LE THYMUS. ANATOMIE—HISTOLOGIE—PHYSIOLOGIE, CLINIQUE ET THÉRAPEUTIQUE. G. Worms, Médecin-Colonel, Professeur du Val-de-Grâce, and H. Pierre Klotz, Interne des hôpitaux de Paris. Price, 30 francs. Pp. 152, with 65 illustrations. Paris: Masson & Cie, 1936.

THÉRAPEUTIQUE MÉDICALE: IX. MALADIES INFECTIEUSES ET PARASITAIRES. M. Loeper. With the collaboration of R. Turpin and of Abrami, Bazy, Debré, Dopter, Dujarric de la Rivière, Joannon, Lemierre, Lesné, Levaditi, Pettit, Tanon. Price, 50 francs. Pp. 415. Paris: Masson & Cie, 1935.